

Responsibility without Government

THE Select Committee on Science and Technology is entirely within its rights to complain, as it did on Wednesday, that the government is dragging its feet on planning for the future of research and development in Britain. In what appears to be a yearning for unattainable perfection, the British Government appears bent on resolving to its own satisfaction and in theory all possible questions before it actually sets out to tackle any one of them. The result is that the report of the committee under Sir Frederick Dainton on the reorganization of civil research, which has already been discussed by the Council for Scientific Policy—its legitimate parent—and passed on to Mrs Margaret Thatcher at the Department of Education and Science, has disappeared into never-never land. Would-be recorders of events have to be satisfied with such straws in the wind as they can find. Does, for example, the fact that the government has made an appointment for only one year as Secretary of the Agricultural Research Council imply that it expects a major reorganization within the year? Time may yet tell, but in the meantime uncertainty persists about the future of the research councils and their relationship with each other, with government departments concerned with specific functions and with the universities especially, because even a rapturous acceptance of the Dainton report would be unlikely radically to transform the functions which research councils at present undertake. There is no need for such a revolution but there is a sense in which procrastination creates problems out of nothing.

The select committee is also entirely right to protest at the way in which the government has done nothing to remove uncertainty, or even to make fuller use of the resources which exist, in the wider context of how civil research as a whole should be undertaken. The Atomic Energy Authority remains in a kind of limbo, with Aldermaston still within the civil field in spite of the near-certainty that it will eventually become a defence laboratory, with the laboratory at Harwell ploughing ahead so successfully at commercial developments of various kinds that it sits increasingly uncomfortably beneath the umbrella of atomic energy, and there is persistent uncertainty not merely about government policy about institutions such as the research associations but towards the whole principle of how and when governments should intervene in industrial research. It may be true, as the government appears to have decided off its own bat, that the time has come for a thoroughgoing review of policy on research. After all, it is now seven years since the report of the Trend committee advocated institutions for regulating the relationship between industry and government on research which have not for practical purposes been taken up (although there is an echo of them in Mr Anthony Wedgwood Benn's now defunct Green Paper on industrial research). It is also true that the issue is important enough to justify a philosophical and measured solution. The question is merely whether industrial research and development can wait that long—and also whether the problem will be the same a year from now.

By all accounts, responsibility for the new policy lies

with Lord Rothschild. At the outset, the Council for Scientific Policy seems to have been fully informed of the status of the Dainton study and in particular of the intention that this should be merely one of several ingredients of a much fuller appraisal of present problems. One of the anxieties now is that a worthy piece of work should be not merely subsumed in a larger study but even made the basis for policy decisions which are put into effect without public discussion in advance. Yet all the experience of the past few decades has made it perfectly plain that juggling with the institutions of the scientific establishment, necessary though it may be, is possible only if the institutions concerned and those who work in them have had a chance to say their piece. On many occasions, the government of the day has been saved from error by precisely such discussion. It would therefore be a token not merely of good intentions but of good sense if the government were now to sanction the publication of the Dainton report. That would be something to be going on with. (At the same time, the Council for Scientific Policy could help its own case for public discussion by helping to ensure that the research councils under its own wing were more forthcoming with the publication of documents of public importance—the report on astronomy in the northern hemisphere which the Scientific Research Council has commissioned (see page 289) should, for example, be published without delay).

100 Years Ago



How, then, did life originate on the Earth? Tracing the physical history of the Earth backwards, on strict dynamical principles, we are brought to a red-hot melted globe on which no life could exist. Hence when the Earth was first fit for life, there was no living thing on it. There were rocks solid and disintegrated, water, air all round, warmed and illuminated by a brilliant Sun, ready to become a garden. Did grass and trees and flowers spring into existence, in all the fulness of ripe beauty, by a fiat of Creative power? or did vegetation, growing up from seed sown, spread and multiply over the whole Earth? Science is bound, by the everlasting law of honour, to face fearlessly every problem which can fairly be presented to it. If a probable solution, consistent with the ordinary course of nature, can be found, we must not invoke an abnormal act of Creative Power. When a lava stream flows down the sides of Vesuvius or Etna it quickly cools and becomes solid; and after a few weeks or years it teems with vegetable and animal life, which for it originated by the transport of seed and ova and by the migration of individual living creatures. When a volcanic island springs up from the sea, and after a few years is found clothed with vegetation, we do not hesitate to assume that seed has been wafted to it through the air, or floated to it on rafts. Is it not possible, and if possible, is it not probable, that the beginning of vegetable life on the earth is to be similarly explained?

From Nature, 4, 269, August 3, 1871



OLD WORLD

SCIENTISTS' PAY

Protests Galore

THE series of meetings organized by the Institution of Professional Civil Servants (IPCS) to publicize the current salary dispute between the British Government and the scientists in its employ is now almost over but the climax is yet to come at the Central Hall, Westminster, on August 10. The support obtained so far has at least demonstrated that government scientists are wholeheartedly behind the IPCS. Whether their anger will be productive remains to be seen.

After the disagreement between the institution and the government, an arbitration tribunal is being set up under the chairmanship of Mr M. J. Mustill, QC. This will sit on August 12 and possibly August 13. A minor success for the institution has been to reverse the original decision to hold the tribunal *in camera* and the proceedings will now be relayed from Abbey Gardens, NW8, to the nearby Bishop Partridge Hall, which the institution hopes to fill with its members.

The final meeting at Central Hall is to be addressed by Dr Harold Turner, of the National Physical Laboratory, Teddington, who is chairman of the institution's Scientific Task Group, and by Mr William McCall, the General Secretary, with Mr J. C. McGloughlan of the IPCS in the chair. The institution is hoping for an overwhelming show of support but at the same time it is not giving any encouragement to the notion being circulated in government laboratories that everybody should take half a day's leave on August 10 in order to attend the meeting—thus effectively bringing all work to a halt on that afternoon. This would violate the promise already given by the IPCS that it would not resort to industrial action to achieve its claims.

A spokesman said earlier this week that the institution is quite optimistic that the outcome of the tribunal will bring a better offer than that already made. The government's view, however, is restated in a letter from the Prime Minister to Mr Airey Neave, the Member of Parliament for Abingdon, who has been taking a close interest in the scientists' claim. Mr Heath has refused to intervene in the dispute and has reiterated that the government had considered all the arguments advanced by the IPCS after it received the first pay offer, but in its view these arguments did not "justify any improvement in the offers that have been made".

A matter of concern to the scientists is the apparent downgrading of their

profession compared with other classes in the Civil Service, in particular the administrative grades who received a salary increase earlier this year. The institution's fear is that there is discrimination against the scientists but Mr Heath states that there is no question of this and "continued progress in science and technology is essential to the future of this country, and the government attaches great importance to the contribution made by the scientific staff within the Civil Service". It is doubtful whether this will mollify the scientists at this stage as the hard facts of the government's offer do not bear out Mr Heath's statement.

Whatever the outcome of the tribunal, the IPCS must consider whether the mechanism at present used to determine salaries within the Scientific Civil Service is the most appropriate. There is no doubt that in view of the latest experience most scientists would prefer not to have their salaries determined as a result of a pay research exercise but some method must be used that will ensure parity with salaries both inside and outside the Civil Service.

BIOLOGY

Old and New at Oxford

OXFORD'S biologists seem to have been celebrating with gay abandon recently. Two weeks ago, the zoologists and psychologists heralded the opening of their new palatial building and on July 24 the Botanic Garden was toasted with champagne on the occasion of its 350th anniversary.



Part of the laboratory built in Oxford for the departments of zoology and psychology. (Photo: Surveyor to the University.)

The garden was founded by Henry Danvers, Earl of Danby, who gave it walls strong enough to withstand the siege of the Civil War and remain unharmed through the succeeding centuries. Many distinguished botanists have tended the plants and worked at their scholarly pursuits within the walls. Although the Professor of Botany no longer personally superintends the activities of the garden, he is still its official keeper, and last week Professor C. D. Darlington, fulfilling one of his last functions before retiring

next September, welcomed the Chancellor of Oxford University, Mr Harold Macmillan, and the guests who had braved the rain to join the celebration. The chancellor marked the occasion by planting a specimen of the scholar tree, *Sophora japonica*.

Among the non-botanical guests on this occasion were Professor J. W. S. Pringle of zoology and Professor L. Weiskrantz of psychology, who recently moved into their new building which was opened by Professor A. L. Hodgkin on July 12. The building, which cost £2 million, was designed by Sir Leslie Martin to bring together two departments which were previously scattered around Oxford. Now they occupy two sides of a complex which is linked by a spine housing common facilities such as libraries and lecture rooms. The furniture and fittings are sumptuous—the workbenches are made of teak; there are two electron microscopes on independently suspended floors; the zoology department is wired for closed circuit television. There is even a tropical climate room.

Brackish Water

THE committee that was set up last year to form an Estuarine and Brackish-Water Biological Association in the United Kingdom has announced plans for the association's inaugural symposium to be held at the Zoological Society of London on October 13, 1971. The need for such an association arises from the varied disciplines of the scientists who are interested in the properties of these waters and so the association will enable people working in such diverse fields as biology and sedimentology to have a common platform. Future plans for the association include regular meetings and the publication of a bulletin.

The committee feels that the essential unity of aquatic science can best be served by emphasizing the function of the previously neglected estuarine sector so that a bridge can be built between the freshwater and marine fields. The formation of the association will then be an important step towards the eventual integration of outlook for these disciplines.

The inauguration of the association will take place during the meeting in October. Membership will be open to anyone but the feeling of the committee is that it will probably be most attractive to research workers in appropriate fields.

ASTRONOMER ROYAL

New Astronomy

A NEW twist occurred last week in the search for a new Astronomer Royal with the announcement that the post is to be dissociated from the directorship of the Royal Greenwich Observatory. This move will please those who have been arguing for just such a break between the two jobs, which will take effect at the end of the year, with the retirement of the present Astronomer Royal and director of the Royal Greenwich Observatory, Sir Richard Woolley.

Now that observational astronomy is no longer the prerogative of optical observatories, the argument goes, it is anachronistic to insist that the Astronomer Royal should also be equipped to manage the optical telescopes associated with the Royal Greenwich Observatory. Indeed, as one astronomer has remarked of the quest for a suitable replacement that has been going on since Sir Richard's retirement was announced a year ago, logically there ought to be astronomers royal for X-ray and radio as well as optical astronomy, a post which, it has been suggested, might have the title of Master of the Queen's Astrophysik.

Such tongue in cheek proposals during the past year have, however, gone hand in hand with real concern that the best decision be made about the role of the Astronomer Royal and of the Royal Greenwich Observatory. One view has been that the post of Astronomer Royal should die when Sir Richard Woolley retires, but most astronomers would probably prefer to keep the old name, either linked to the Royal Greenwich Observatory or as an honorary title that can be held by any British astronomer, perhaps for a set period only.

Whether last week's announcement will make any easier the task of finding a successor to fill Sir Richard Woolley's second role as director of the Royal Greenwich Observatory is a debatable point. The overt explanation for separating the two posts is that it throws open the directorship to foreign astronomers—the Astronomer Royal cannot be non-British, although oddly enough the post of Astronomer Royal for Scotland has only once been held by a Scot. But Sir Brian Flowers may now find that some potential candidates will be discouraged by what amounts to a downgrading of the post. It is believed that a Dutch astronomer has already been unsuccessfully approached.

More important than the personality of the next Astronomer Royal, however, is that this watershed is being taken as an opportunity for a reappraisal of British astronomy. The time

is particularly appropriate, for during the next few years many of the grand old names of British astronomy will reach retiring age, so that several crucial chairs will become vacant. To the extent that the Science Research Council can influence appointments within the universities, decisions taken during the next few years will affect British astronomy for several decades. Traditionally the Royal Greenwich Observatory has been at the heart of British astronomy, but since the war the new branches of astronomy that have developed outside the Royal Observatory system have called into question the wisdom of giving such a prominent role to an organization that was established primarily to provide astronomical information for mariners. This question came to a head a year ago when the Science Research Council was handed a review of the future of astronomy in the northern hemisphere that it had commissioned from a group of scientists that included astronomers, a space scientist, a nuclear physicist, and the two astronomers royal. As usual the report is being treated in a typically close-handed way by the Science Research Council, and is now unlikely to be published.

It is, however, understood that the most contentious proposal is that a British observatory to be established in the Mediterranean area be managed not by the Royal Greenwich Observatory, but by a new institution that would be linked with a group of universities. An association similar to the federation of ten universities that manages the Kitt Peak observatory in the United States is what the review committee had in mind, and is thought to have been accepted by all members of the committee with the exception of the two astronomers royal.

Although on paper at least the Royal Greenwich Observatory does not have control of the only large telescope that is available to British astronomers—the 98-inch Isaac Newton telescope—or the 150-inch telescope that is being built as a joint venture with the Australians, there seems to be a widespread feeling that the observatory exerts an influence on the running of the telescopes that is inimical to the progress of British astronomy.

In any case, the staff at the Royal Greenwich Observatory saw how the wind was blowing as long ago as November 1968—before the northern hemisphere review began in earnest—when all the scientific staff of the observatory signed a petition to the Queen to seek assurances that the directorship of the observatory would continue to be linked with the post of Astronomer Royal. At about the same time, Sir Brian Flowers wrote to the staff at the observatory saying that the

staff would be consulted if there were any proposal to dissociate the directorship from the post of Astronomer Royal, a promise which the Institution of Professional Civil Servants says has not been kept.

In other words, the staff of the observatory are rightly concerned that the announcement last week marks the beginning of a downgrading of the observatory. The staff expect the first consequence to be that greater difficulty will be experienced in finding a strong candidate for the directorship. But at the backs of their minds is the fear that the Science Research Council will encourage the Royal Greenwich Observatory to become a service organization, at the behest of the universities.

Views on the appropriateness of such a future for the observatory depend on what attitude is taken to the usefulness of scientific institutions that are divorced from the universities and from industry (although the staff of the observatory will point to their strong links with the vigorous astronomy department at the University of Sussex). But for the time being at least, the plan to set up a new institution linked with a number of universities appears to have been shelved, apparently because of the cost although this is thought to have been modest. On the face of it, then, the present arrangements for managing the large optical telescopes will continue. Nevertheless, the next director of the Royal Greenwich Observatory will have to be prepared for criticism of the establishment from many astronomers who see the observatory as a white elephant. In particular, the £1.2 million which the Science Research Council spends on the Royal Greenwich Observatory, the Royal Observatory at Edinburgh, and on the observatories in South Africa is widely held to give poor returns compared with the running costs of the Hale Observatories of \$1.7 million per year. In addition, the siting of the 98-inch Isaac Newton telescope adjacent to the Royal Greenwich Observatory a mile or so from the Sussex coast is still felt to be the most short-sighted decision in the management of British astronomy. The small amount of astronomy that it has been possible to carry out with the telescope compares unfavourably with the productivity of the Hale Observatories, for example, and many astronomers would be happy to see the telescope moved to the Mediterranean area at the first opportunity. It is true that there is little hope now that a site can be found in the Mediterranean that will produce the rapturous praise that astronomers reserve for the skies of Chile, but almost anywhere, the saying goes, will be better than Sussex.

NEW WORLD

AEC Bruised but not Cut

by our Washington Correspondent

AFTER the Food and Drug Administration, the Atomic Energy Commission probably ranks second in the list of the most heavily criticized federal agencies. During the past year it has had to face a barrage of criticism from environmentalists who oppose extension of the nuclear power programme, its safety procedures have been thrown into disrepute by a series of failures under test of the emergency core cooling device, and it has lately been placed in a compromising position by its advocacy of the nuclear test planned to take place in Alaska in the autumn.

This outspoken public disenchantment with the AEC's plans has not, however, gained a footing in Congress, for only last week both houses agreed to authorize more funds for the AEC than were requested in its budget, and only a few minor programmes have been trimmed by the Joint Committee on Atomic Energy which recommended the authorizations to Congress. Although the authorizations represent only the maximum funds that can be appropriated for the commission—its budget must now be considered by the Appropriations Committee—it seems likely that the AEC will be able to proceed with all the major parts of its planned programme for 1972.

The authorizations passed by Congress amounted to just over \$2,321 million, \$37 million more than the commission requested, and essentially the same amount as authorized for 1971. Chief beneficiaries of this Congressional largesse are the programmes for nuclear space propulsion (the Nerva rocket programme) and gaseous diffusion technology, which were increased by \$37 million and \$31 million respectively. The Joint Committee on Atomic Energy has, however, docked \$21.5 million from the AEC's request for funds to establish a radioactive waste repository at Lyons in Kansas, and cut by half the commission's request for \$1 million to develop a nuclear powered cardiac pacemaker, although the full amount was later restored by an amendment which was proposed in the Senate by Hugh Scott, the ranking Republican from Pennsylvania.

The joint committee left untouched the \$263.6 million requested for physical research, although it has a cautionary word to say to the high energy physics laboratories operated by the AEC. When the 200 GeV accelerator at the National Accelerator Laboratory in Illinois becomes fully operational, it will require an annual funding of between \$60 and \$70 million which is more than half the \$116 million requested for the whole high energy physics programme for 1972. The joint committee therefore asks the AEC to "examine the minimum level of support necessary to keep each of its high energy accelerator laboratories, including the NAL, viable and productive, and that it develops a priority listing of which accelerators should be kept operating should future funding be less than the minimum necessary to effectively support each of the six laboratories". The AEC has been asked to furnish the joint committee with its priority listing by the end of 1971.

Following the lead of the Senate Committee on Aeronautical and Space Sciences and the House Committee on Science and Astronautics, the Joint Committee on Atomic Energy has restored in full the funds requested for development of the Nerva nuclear powered rocket. In its budget request to the Administration, the AEC asked for \$52 million for research and development on Nerva, but this was slashed by the Office of Management and Budget to \$15 million—barely sufficient to keep the project ticking over, and probably a sufficient reduction to kill the project altogether.

What impressed the joint committee most was a series of joint hearings conducted with the Committee on Aeronautical and Space Sciences in February, during which a series of witnesses testified that the Nerva programme has so far been successful and is up to schedule. The joint committee therefore proposed, and Congress agreed without criticism, that the AEC should get the full \$52 million requested, and that this should be split into \$35.2 million for rocket propulsion development, \$13.8 million for advanced research and development,

and \$3 million for operation of the Nuclear Rocket Development Station in Nevada (the Administration had deleted all funding for this latter facility).

The joint committee also looked favourably on Project Plowshare—the proposal to excavate natural gas by nuclear explosives. Congress last week authorized \$8.1 million for this project—an increase of \$3.1 million on the Administration's request. The idea is to use nuclear explosives to release natural gas from underground deposits more cheaply than by conventional means, but this proposal has already fallen foul of many sectors of opinion. Dr John Gofman, an ardent critic of the Atomic Energy Commission, for example, said last week that he regards the decision to proceed with Plowshare as "extremely unfortunate". Pointing out that "all these programmes eventually end up spewing radioactivity into the atmosphere", Gofman said that the concept that natural gas can be excavated by nuclear explosives and pumped into people's homes along with some radioactive debris is "so grotesque that we should have abandoned this project long ago".

The AEC estimates that Project Plowshare can bring natural gas to the consumer pipelines within five years, at an estimated government cost of \$50 million, and to this end it proposes to test a device in 1972. If successful, it has been estimated that the project will double the nation's natural gas reserves, and apart from the economic advantages of such an achievement, its supporters justify Plowshare on the grounds that it will provide the market with a largely pollution-free fuel. But that justification would be something of a mockery if the project ended up contaminating the atmosphere with radioactivity, and if it also provided natural gas contaminated with radioactivity.

The joint committee has, however, sounded a more cautionary note in its deliberations on the AEC's proposals for the long term storage of radioactive wastes. The kingpin of the AEC's programme is to store such wastes in a disused salt mine in Lyons,

Kansas, and the commission requested \$25 million for acquisition of land and for construction of facilities. But the joint committee has slashed this request by \$21.5 million. In spite of a report by the National Academy of Sciences, which concluded that the use of bedded salt deposits represents the safest disposal method now available, the joint committee has taken the sensible step of requiring that the AEC makes further studies of the safety of the mines before they are acquired for use. In particular, the joint committee has asked for more information on heat transfer between the salt/shale interface, areal hydrology and geology, possible environmental damage, the transportation of waste to the salt mine and waste retrieval after burial. The AEC has, however, stated that such questions cannot be answered until small amounts of waste have been deposited in the mine, but that argument cut no ice with the committee, which stated in its report to Congress that "all reasonable questions raised must be answered from the standpoint of satisfying Kansas and Federal officials *before the Lyons facility is put into operation as a repository for industrial wastes*". (Committee's italics.)

The most controversial item in the AEC authorizations is the \$19.7 million requested for the testing of a nuclear weapon in an underground site on Amchitka Island in the Aleutians off the coast of Alaska. The reason for the test, which was explained by the AEC in full only a day or so before the Senate debate on the authorizations, is to monitor the X-ray spectrum produced by the warhead of the Spartan anti-ballistic missile (the longer range missile that forms part of the Safeguard system). A statement sent by the AEC to Senator John O. Pastore, chairman of the Joint Committee on Atomic Energy, pointed out that the kill mechanism of the Spartan missile is X-rays released outside the atmosphere, and that this requires a low fission output so as to minimize radar blackout, and a high X-ray output.

The AEC therefore uses a mixture of economic, scientific and pragmatic justifications for the test. It says, for example, that failure to conduct the test would drastically reduce confidence in the missile, and that the test is necessary "to minimize the possibility of stockpiling a defective design", to measure the fission output, and to monitor the X-ray flux and yield. If the test does not take place, the AEC points out, "one would be forced to rely on theoretical calculations to determine X-ray flux and spectrum without the benefit of any meaningful experimental data".

Code-named Cannikin, the test is due to take place in October. With a yield of five megatons, it will be the largest underground explosion ever conducted by the United States, and it has already sparked off a wealth of political and scientific criticism. But amendments to the Authorizations Bill, introduced in the House by Mrs Patsy Mink, and in the Senate by Senator Mike Gravel, failed to prevent funds being authorized for the explosion, and the chief hope now is that President Nixon will veto the programme. Opposition centres around four chief points: that the test will release radioactive debris into the atmosphere directly through venting, and later by seepage of ground water; that the explosion may trigger an earthquake which could cause a tidal wave, resulting in substantial damage along the Pacific coast; that it could sabotage the Strategic Arms Limitation Talks (SALT) now taking place between the United States and the Soviet Union in Helsinki, and that in any case development of the Safeguard ABM system is likely to be a destabilizing influence on the arms race.

As far as the environmental consequences of the test are concerned, the AEC has consistently underplayed the likely effects of Cannikin. To be sure, even critics of the test acknowledge the fact that dire environmental consequences are a remote possibility, but they also point out that as long as the possibility exists the test should be scrapped. The most publicized risk involved in the test is that it may trigger off a massive earthquake in the region which could in turn cause a tidal wave, with devastating consequences for the Pacific islands, and even for California and other parts of the US Pacific coastline. The Aleutian islands are a particularly active earthquake area, and it has been estimated that the Cannikin blast will be equivalent to an earthquake of magnitude 7 on the Richter scale. (By comparison, the quake which shook California earlier this year was rated at 5.9.)

In an environmental policy statement which the AEC was required to publish under the terms of the National Environmental Policy Act of 1969, however, the commission stated "experience gained by observation of past nuclear detonations and earthquakes combines to provide extremely positive assurance that the Cannikin explosion cannot of itself cause a severe earthquake". But an *ad hoc* panel of scientists headed by Dr Kenneth Pitzer, which met under the auspices of the Office of Science and Technology in 1968, took a less rosy view of the situation. Its report stated: "There does not now appear to be a basis for eliminating the possibility that a large test explosion might induce, either immediately or after a period of

time, a severe earthquake of sufficiently large magnitude to cause serious damage well beyond the limits of the test site. This possibility is more serious for tests greater than a megaton. . .". It has also been suggested that there is a one in a hundred chance that an earthquake bigger than the Cannikin blast will occur in the Aleutians in any two week period. What would happen if such an earthquake occurred simultaneously with the Cannikin test is a moot point.

Two underground tests conducted at the Nevada site have also been found to release more than ten times the energy released by the blasts themselves. But the Pitzer report pointed out: "Since the Amchitka area in Alaska is still more active seismically, the hazard of inducing an earthquake must be considered to be greater at that location than at either Nevada site." One point that the AEC emphasizes, however, is that the Milrow test—a one megaton "proving" test for the Cannikin blast, conducted at Amchitka in 1969—did not produce any sizable after-shock.

As far as leakage of radioactive debris is concerned, one fear which was suggested last week by Dr Robert Fleisher, director of the Committee for Nuclear Responsibility, is that if massive venting does occur (and some of the Nevada tests have resulted in quite large venting), radioactive fallout could be carried outside United States territory, in violation of the partial test ban treaty. There is also a fear that if there is an unsuspected fault close to the test site, leakage of radioactive material through groundwater could occur relatively quickly, resulting in danger not only to the wildlife in the Northern Pacific, but also to the Alaska coast. And this fear is intensified by suggestions that the Cannikin blast could also release radioactive debris remaining from the Milrow test. The AEC's environmental policy statement suggested, however, that "it is not conceivable that Cannikin would cause release of underground radioactivity from the cavities which remain from the earlier Milrow and Long Shot tests". Unfortunately, however, that statement was not accompanied by any discussion of why such a possibility is inconceivable.

In view of these serious doubts about the safety of the test, the Committee for Nuclear Responsibility, whose board of directors contains the names of James Watson, John Gofman, Paul Erlich, Linus Pauling and George Wald, has filed a suit against the Atomic Energy Commission, in an attempt to stop the test. Dr Robert Fleisher, executive director of the committee, said last week that the National Environmental Policy Act

requires that if a project involves the possibility of serious ecological damage, it must be proved to fulfil an enormous need. He charges that the AEC's environmental policy statement does not provide such evidence, and that will be the basis of the committee's court action.

The most likely possibility for stopping the test rests, however, in its political repercussions. For one thing, it could seriously damage the chances of success in the SALT talks, which according to recent reports are progressing along hopeful lines. It has been suggested, for example, that the United States has proposed verbally, and will later propose in writing, an agreement that would halt the construction of both land-based and sea-based missiles, and also that as many as 300 missiles would be allowed in the US and the USSR to protect offensive missile sites. For President Nixon to cancel the Cannikin test would therefore help to establish his credibility in his desire to curb the arms race.

But a more immediate factor is the Presidential elections in 1972. Now that the possible consequences of the Cannikin blast have been aired in the press and in Congress, Nixon could well put off the test until after the 1972 campaign. If he in fact decided to go ahead on schedule, and an unlikely catastrophe did take place, his chances of re-election could be ruined. Some observers are very optimistic about the chances of a Presidential veto, and one critic of the programme suggested last week that the chances are "fifty-fifty or better". Another but less likely possibility is that the appropriations for the test could be deleted when they come up for debate in Congress. Senator Gravel is likely to oppose them in the Senate, and he will probably get more support than he did last week when he proposed an amendment to the Authorizations Bill seeking to delay the test until after May 31, 1972. That amendment was defeated by a vote of 37 to 57, but with more exposure in the press, and if the SALT talks continue to progress towards a moratorium on the construction of nuclear weapons, Gravel may be able to muster enough votes, if not to defeat the appropriations outright, at least to provide Nixon with added justification for vetoing the measure.

FDA

New Name, Old Face

by our Washington Correspondent

IF the Administration gets its way with Congress, the name of the Food and Drug Administration may soon be deleted from the list of Federal agencies, and in its place will be sub-

stituted the much more grandiose title of the Consumer Safety Administration. But this exercise in semantics will do little to appease the many critics of the way the FDA conducts its business, for instead of radical changes in the functioning of the FDA, what is being proposed is that it should have extra responsibilities grafted on to its present unsatisfactory structure. The proposal, in the form of a bill introduced into the Senate by the Administration, came under scrutiny by the Senate Commerce Committee last week, and it has also been given President Nixon's personal blessing.

The idea is that the FDA will cease to be solely a watchdog over food and drugs and that instead its responsibilities should extend over the whole field of product safety. This proposal was spelt out in some detail by Elliot L. Richardson, Secretary of Health, Education and Welfare, before the Commerce Committee last week. He said that when the bill is passed, "I propose to build upon the FDA framework by establishing a new Consumer Safety Administration to be composed of three major offices: the Office of Product Safety Regulation, the Office of Drug Regulation and the Office of Food Regulation." And, in a letter to Senator Frank E. Moss of Utah, chairman of the subcommittee considering the bill, Nixon left no doubt about the fact that the FDA will continue to operate in much its present form. After announcing his approval for the bill, the letter went on to say that "this unit (the proposed Consumer Safety Administration) will build upon the activities, personnel and facilities of the FDA, which has a long and distinguished history in the field of consumer safety".

The Administration's bill is a counter proposal to a bill introduced by Senator Moss and Senator Warren G. Magnuson which also seeks to extend the Federal Government's responsibility for the control of potentially dangerous consumer products (such as toys that are likely to cause actual bodily harm), but the essential difference between the two proposals is that the Moss-Magnuson bill seeks to set up a division of product safety outside the Department of Health, Education and Welfare. The chief idea behind such a measure is that the new agency could respond more readily to consumer interests if it were taken out of the bureaucracy of HEW, and it stems from a report published by the National Commission on Product Safety. Testifying before the Senate Committee on Commerce last week, David Elkind, Chairman of the National Commission on Product Safety, left no doubts about his dislike for the Administration proposal, calling the proposed Consumer Safety

Administration a "paper tiger", Elkind said "the first step in creating a paralytic consumer protection function is to place it deep in the recesses of the already overburdened and unsuccessful Department of Health, Education and Welfare".

Why has the Administration chosen to oppose the Moss-Magnuson bill? Apart from the fact that to set up a unit outside the FDA may be regarded as the first step in dismantling the agency, the overt reason is one of administrative neatness. Richardson, for example, said that the FDA already has extensive laboratory and testing facilities, and that to set up a separate organization outside HEW may duplicate some of those facilities. He dismissed the idea, moreover, that an independent agency would be more receptive to consumer pressures than an expanded FDA, and came close to agreeing with critics of the FDA when he suggested, "I do not think that independent regulatory agencies have historically shown a greater resistance than other agencies to the pressures that often cause agencies to identify their interests with those of the industries they regulate".

If the Commerce Committee eventually decides not to go along with the Administration, but to support the Moss-Magnuson bill, the FDA would find itself in an embarrassing position, for Richardson confessed to the committee in reply to a question from Moss that he has already set up a division of product safety within the FDA, and Dr Charles Edwards, Commissioner of the FDA, later explained that in three to five years' time the Office of Product Safety would be the largest division of the FDA. He suggested that \$19 million would be spent on setting up the new office in 1972, and that some of this money would be taken from the already undernourished and overburdened food and drugs offices.

According to Edwards, the new style FDA will parallel the Environmental Protection Agency in terms of function of operation, but Elkind was quick to point out that the essential difference between the two agencies is that the FDA will continue to operate from within the bureaucracy of the Department of Health, Education and Welfare, while the EPA was set up as an independent agency for the very reasons that the National Commission on Product Safety asked for an independent Consumer Safety Agency. The Administration's bill, he retorted, would "be as though the government were to fashion, like Dr Frankenstein, a man with beautiful sinews, long arms and piercing eyes, with just one weakness—an inability to hear from the consumer and respond to his needs".

NEWS AND VIEWS

X-Chromosome Inactivation in Mules and Hinnies

ACCORDING to the inactive-*X* hypothesis of dosage compensation (Lyon, *Nature*, **190**, 372; 1962), one or other of the two *X*-chromosomes of the mammalian female becomes genetically inactivated at an early stage of embryonic development and the choice of which *X* becomes inactivated in each cell is a random one, with the result that clones of cells arise within individual females, having either the maternally-derived or paternally-derived *X* in the inactive condition. The late DNA replication of one *X*, observed in the somatic cells of female mammals, and genetic inactivity of one *X* are generally considered to be related phenomena but as yet there is no final proof of this.

Horse-donkey hybrids have two qualities which make them unique material for testing the inactive *X* hypothesis: first, the horse and donkey *X*-chromosomes are morphologically distinguishable and, second, the *X*-linked genes which code for glucose-6-phosphate dehydrogenase (G6PD) in each species show different electrophoretic mobilities. A mixed expression of horse and donkey G6PD is generally found in cell cultures from female mules and hinnies but evidence that this derives from the presence of two cell populations, one with the donkey *X* active and one with horse *X* active, has only now been obtained. Hamerton, Gianelli and co-workers in an article on page 349 of this issue of *Nature* have shown that cloning cells from original skin fibroblasts which show mixed G6PD expression yield clones which produce either horse or donkey G6PD. A small proportion of clones showing mixed expression were also found but, on recloning, these could again be broken down into the two component cell types. These data are thus consistent with the inactive-*X* hypothesis but, although they do not disprove Grüneberg's hypothesis (*J. Embryol. Exp. Morph.*, **22**, 145; 1969) that the activity of one or other *X* may range from 0 per cent to 100 per cent of the total *X*-chromosome activity of any one cell, they make it seem most improbable that such a mechanism operates in the mule, at least *in vitro*.

An impressive inverse correlation between late replication of the horse and donkey *X*-chromosomes and horse and donkey G6PD expression has recently been demonstrated by Cohen and Rattuzzi in the female mule (*Proc. US Nat. Acad. Sci.*, **68**, 544; 1971) and the two articles by Hamerton and Giannelli on pages 312 and 315 of this issue of *Nature* amplify these findings. With both the mule and the hinny, agreement was found between the frequency of cells in original fibroblast cultures with late replicating donkey or horse *X*-chromosome and the estimated frequency of cells in these cultures expressing donkey or horse G6PD, as determined by the yield of the two cell types in cloning experiments. Final proof of a definite relationship between late replication and genetic inactivity will be obtainable when late replication of the *X* is studied in horse and donkey G6PD clones.

Perhaps the most intriguing information provided by the present studies on horse-donkey hybrids is the discovery of extensive cell selection operating both *in vitro* and *in vivo*. In the *in vitro* studies this was best seen from the results of recloning mixed clones. If it is assumed that these mixed clones originated from two cells, one with the horse *X* active and one with the donkey *X* active, the proportion of sub-clones expressing the donkey G6PD is found to be significantly lower than that expected. Whereas this observation suggests powerful selective forces acting against cells with the donkey *X* active, it would be useful to subject this interpretation to test. This could be done by cloning cultures derived from artificial mixtures of the two cell types.

Hook and Brustman's article on page 349 provides striking evidence of organ differences in G6PD expression among a very large sample of female mules. Two organ types could be identified, one in which the horse G6PD predominates and one in which horse and donkey G6PD expression is not significantly different from that expected on a random basis. Because a predominance of donkey G6PD was never consistently found in any tissue studied, these data agree with Hamerton's in suggesting that selective forces operate against cells with the donkey *X* active, at least in some tissues and organs.

Earlier studies on the female mule had shown little agreement as to the frequency with which the horse and donkey *X*-chromosomes show late replication. In the light of the new evidence it seems most probable that this only reflected inter-animal variation. This could result either from the randomness with which one or other *X* is inactivated and/or from differences in the selection forces operating upon the two cell populations in different animals. In this context it will be interesting to see if selection operates against cells with a donkey *X* active even in animals which show a predominantly donkey G6PD expression. The new data do not contribute any evidence on the randomness of the *X* inactivation process in horse-donkey hybrids though they rule out the once real possibility that the paternally-derived *X* might be preferentially inactivated in the hybrid of each reciprocal cross.

New Class of Varying Stars

FOLLOWING the discovery of X-ray pulsations from Cygnus X-1, the data collected by the Explorer 42 X-ray satellite (Uhuru) have been intensively searched for evidence of any similar pulsations in other sources. To the surprise of most astronomers—and probably to their own surprise as well—R. Giacconi, H. Gursky, E. Kellog, E. Schreier and H. Tananbaum have now found that the source Centaurus

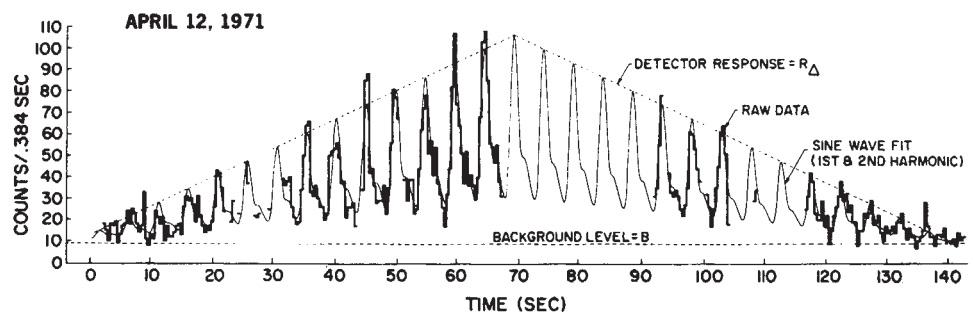
X-3 varies dramatically with a period of nearly 5 s, as shown in the figure. These variations are markedly different from the variations seen in pulsars, quite apart from the fact that in both Cyg X-1 and Cen X-2 there is no evidence of associated optical and radio pulsations (*Astrophys. J. Lett.*, **167**, L67; 1971).

The Cyg X-1 discovery itself caused quite a stir among astronomers and although this source has now been identified at radio frequencies (Braes and Miley, *Nature*, **232**, 246; 1971) it remains difficult to fit it into the accepted pulsar model. But the Cen X-3 discovery is even more remarkable, and although many people would be prepared to argue that the evidence for periodic variations in the X-ray flux from Cyg X-1 is so marginal that it could be discounted, it would be a brave man who ventured to

model, might be revived to explain this newly discovered phenomenon.

So Cen X-3, and in all probability Cyg X-1, cannot be fitted to the model of pulsars built up over the past three years. The sudden changes in period, the presence of overtones, and the simple fact that no optical or radio pulses are seen all point to the same conclusion. Do these sources form a new class of previously undetected objects? Or are they simply a previously unexpected manifestation of the pulsar phenomenon? In either case, there is a great deal of hard thinking ahead of the theoreticians. It may even be that Cen X-3 and Cyg X-1, as well as being different from pulsars, are entirely different sorts of object from each other. Indeed, the apparent differences between the spectra, Cyg X-1 following a power law and Cen X-3

X-ray variations of Centaurus X-3. Roughly 70 per cent of the power radiated at 2-6 keV is pulsed. From *Astrophys. J. Lett.*, **167**, L67; 1971).



suggest that the period seen in the flux from Cen X-3 is not a genuine effect (compare the figure here with that in *Nature Physical Science*, **231**, 91; 1971). The problem of the origin of these periodicities remains unsolved, but it is becoming increasingly clear that they cannot be interpreted in terms of the rotating neutron star model of pulsars.

In their latest report, Giacconi *et al.* discuss variations in the flux from Cen X-3 with time scales of days, minutes and seconds. Although the intensity of the source changed by a factor of 10 over January 11 and 12 of this year, the mean flux before and after this increase was remarkably constant. The almost sinusoidal rapid variation which is seen after this flaring has a period of 4.8713 s for the first 9,000 s after the flare, which changes over the next hour to a 4.8703 s period, this in turn lasting for roughly 28,000 s before changing, again over an hour, to a 4.8726 s period. This decrease by 1 ms followed by an increase by 2.3 ms poses serious problems in constructing models of the source. Furthermore, by April, the period of the variation had decreased by roughly 30 ms, that is, by 0.6 per cent.

In carrying out a search for evidence of any more rapid variation, the team have found only the "fundamental" 4.8 s period and a strong "first harmonic" at 2.4 s, using Fourier analysis of the January data. Any other rapid variations must occur with a time scale that is short compared with the 0.096 s integration time of the detector used. The presence of two harmonics in the variation, together with the sinusoidal shape of the curve which best fits the observed changes, also points to a pulsational origin, rather than one associated with rotation. Perhaps the pulsating white dwarf model of pulsars, discarded by theoreticians in favour of the rotating neutron star

seeming more like a blackbody, make it very likely that the sources have different origins.

Just as the development of optical astronomy earlier this century opened up unforeseen areas of research and led to the development of theories explaining the early and middle stages of stellar evolution, so high energy astronomy is now revealing equally unprecedented data, which may, when gathered into a coherent pattern, reveal the way in which stars move towards the end point of their evolution, producing short lived energetic phenomena such as pulsars and at least one or two types of regularly variable X-ray stars before they sink into obscurity as cold white dwarfs or neutron stars.

Confusion over Fossil Man

WHEN Leakey, Tobias and Napier redefined the genus *Homo* in 1964 they also described the type of the earliest known species of this genus, *Homo "habilis"*. The redefined genus as well as the new species have been the object of considerable discussion and dispute and the attribution of Olduvai Hominid 24 to *Homo habilis* by Leakey, Clarke and Leakey on page 308 of this issue of *Nature* will in no way lessen the controversy. The revised definition of the genus *Homo* included several cranial features that differed only slightly from, or overlapped with, those found in *Australopithecus*; indeed, many of the cranial criteria were so ambiguously flexible that perhaps only the odd gorilla would have been denied membership. It must be pointed out, however, that distinctions between genera are not subtle and overlapping features, but they

reflect and represent major adaptations to a particular way of life; that is the "adaptive plateau" discussed by Sewall Wright, Ernst Mayr and others. It is difficult, therefore, for some palaeo-anthropologists to accept as generically distinctive the differences in certain features in *Australopithecus africanus* and *Homo "habilis"* which are probably due only to normal variation within a single species. Moreover, the slow evolution of a single, widespread population of *Australopithecus* into *Homo* would seem more consistent with modern evolutionary theory than with the continued existence of several generically and specifically distinct groups of hominids.

Criticisms of theory aside, it is interesting and surprising to find that the new *Homo "habilis"* from Olduvai does not fulfil all the generic criteria outlined in the 1964 revision. The revision calls for a minimum cranial capacity of "about 600 cm³". Although some gorillas have more than 600 cm³, Olduvai Hominid 24 has only 560 cm³. This is admittedly a preliminary estimate because there is "still some evidence of distortion in the reconstruction" and two inevitable questions arise: if cranial capacity cannot be accurately determined does this not undermine the validity of many of the cranial measurements? And does it not also undermine the validity of the conclusions that are drawn, at least in part, from those measurements?

One taxonomic criterion included in the 1964 revision was not phrased in equivocal terms: a member of the genus *Homo* will not have the concave or "dished" face characteristic of all known australopithecines. The dished face is a composite feature of considerable evolutionary significance and its absence in later hominines represents a diminution of the teeth and jaws, with reduced prognathism in the lower part of the face and emergence of the nose. The profile line drawing (Fig. 3 on page 310) clearly demonstrates the dished face of Olduvai Hominid 24 and serves to emphasize its overall similarity with the undoubted australopithecine from South Africa.

Olduvai Hominid 24 has been nicknamed "Twiggy" because its flattened shape before restoration resembled that notably unendowed figure. A badly crushed and distorted skull nearly two million years old presents some problems to those restoring it; it presents even more problems to those who try to decide, finally, what it is. On the basis of the evidence presented by Dr Leakey it is very difficult to agree that this specimen is "clearly *Homo*". Indeed it would seem to be excluded from that group on the basis of his own criteria are outlined in his 1964 article.

TRANSFORMATION

Responses to Cyclic AMP

from our Cell Biology Correspondent
EARLIER this year I drew attention in this column to the work of Puck and his associates and Pastan's group who respectively showed that dibutyryl adenosine cyclic 3',5' monophosphate causes Chinese hamster ovary cells growing in culture to assume reversibly a fibroblastic morphology, and causes the phenotypic reversion of transformed

mouse fibroblasts. More recently *Nature's* Cell Physiology Correspondent (*Nature New Biology*, 232, 35; 1971) commented upon the experiments of Sheppard, who has shown that doses of this substituted cyclic AMP not only restore the growth control and susceptibility to contact inhibition of populations of 3T6 cells and 3T3 cells transformed by polyoma virus but also reduce the susceptibility of these cells to agglutination by wheat germ agglutinin.

Myoglobin Sequence Completed

THE torrent of articles concerned, on the one hand, with the structure and function of haemoglobins and on the other with the characterization of variant haemoglobins, shows no sign of abating. A welcome addition, however, is the appearance, in next Wednesday's *Nature New Biology*, of three articles on the sequence of human myoglobin, the choice of an allosteric model for the haemoglobin-oxygen system, and the relation between structure and properties of the Japanese human haemoglobin variant Hb-Hiroshima.

Herra and Lehmann have repeated some parts of Hill's sequence studies on human myoglobin and disagree with him on only three points. They have also completed the sequence which largely agrees with that proposed from considerations of amino-acid composition and homology with the known sequence of sperm whale myoglobin. Their sequence allows the differences between seven myoglobins to be delineated and will no doubt stimulate discussion on the homology and evolution of mammalian myoglobins. The unique feature of human myoglobin is a cysteine residue at position 110 (G11), which it has in common with the α -chain of human haemoglobin. Apart from this feature, human myoglobin resembles the human α -chain to no greater extent than the β -chain, having thirty-seven identical residues with each. Of these several are discovered among the haem contacts of the α and β -chains.

The complete human myoglobin sequence will be of great value in determining the structural differences of the four known human skeletal myoglobin electrophoretic variants; the possible existence of neutral mutations can also be investigated. Herra and Lehmann report no differences in the fingerprints of human skeletal and heart myoglobin and the skeletal myoglobin of a case of Duchenne's muscular dystrophy. Further work on human myoglobin variants, which has in the past been an area of some confusion, will be awaited with interest.

Connoisseurs of theories of allosteric systems will no doubt appreciate a re-examination by Minton in next Wednesday's *Nature New Biology* of a recent treatment by Edelstein (*Nature*, 230, 224; 1971) of the allosteric model for haemoglobin. Edelstein had concluded that the sequential model of Koshland, Nemethy and Filmer describes the oxygen binding curves of several species of human haemoglobin less well than does the two-state model of Monod, Wyman and Changeux. Minton points out that a tacit assumption in Edelstein's analysis is likely to be invalid, in which case it cannot be shown that any one of the two models is more appropriate to describe the haemoglobin-oxygen system.

In another article, Perutz and Shibata and their co-workers have re-investigated the Japanese haemoglobin variant Hb-Hiroshima, in which the magnitude of the Bohr effect is halved, the oxygen affinity at physiological pH is increased three times and the haem-haem interaction is somewhat reduced. This variant was thought to have the substitution $\beta 143$ (histidine $\rightarrow$ aspartic acid), but the normal effect of 2,3-DPG on the oxygen affinity of Hb-Hiroshima was inconsistent with the proposed role of this residue in the binding of 2,3-DPG by normal adult haemoglobin.

Perutz and his colleagues have now been able to show, both by X-ray crystallography of deoxy Hb-Hiroshima and by chemical methods, that the replacement is in position $\beta 146$. The physiological properties of Hb-Hiroshima can now be accounted for without difficulty. The result also confirms the earlier suggestion that half the alkaline Bohr effect is due to the salt bridge between the imidazole side chain of $\beta 146$ (His) and the γ -carboxyl group of $\beta 94$ (Asp). The reduced haem-haem interaction in Hb-Hiroshima is also related to the absence of a histidine residue at $\beta 146$, which affects both the tertiary structure of the β -chains and the quaternary structure of the entire molecule.

In the latest issue of the *Proceedings of the US National Academy of Sciences* (68, 1648; 1971), Hsie, Jones and Puck describe their latest observations of Chinese hamster ovary cells exposed to dibutyryl cyclic AMP alone or in combination with a variety of steroid and other hormones. The change in morphology and pattern of growth of these cells on exposure to this substituted cyclic AMP is accompanied by a strict susceptibility to contact inhibition of growth and a decreased susceptibility to agglutination by wheat germ agglutinin. Moreover, in the presence of dibutyryl cyclic AMP Chinese hamster ovary cells fail to round up when exposed to concanavalin A or antibody lacking complement. The synthesis of collagen as judged by the intracellular concentration of hydroxyproline, which is of course a characteristic property of fibroblasts the expression of which may be modulated by virus transformation, is, however, increased six to ten times when these ovary cells are exposed to the drug.

Puck and his colleagues have also tested a wide range of male and female steroid sex hormones to discover whether the synergistic effect of testosterone on the morphological conversion induced by dibutyryl cyclic AMP is highly specific or common to many if not all steroids. They found that the male steroid hormones and some prostaglandins all have this property but the steroid oestrogens do not. Apparently the group have isolated mutant cells which have a fibroblastic morphology even in the absence of cyclic AMP; they believe that these cells carry single gene mutations and their analysis—an exciting prospect—should turn attention to the genetic control of morphological and biochemical transformation induced by this unique nucleotide.

It would be wrong, however, to run away with the idea that the multiplication of all cells is inhibited, or at least brought under strict contact inhibitory control, by cyclic AMP. For MacManus, Whitfield and Youdale, in an interesting article published earlier this year (*J. Cell Physiol.*, 77, 103; 1971), reported that when rat thymic lymphocytes are exposed to epinephrine (adrenaline) the intracellular concentration of cyclic AMP and the activity of adenyl cyclase are both increased. And in due season these changes are followed by the induction of DNA synthesis and mitosis.

In other words, although cyclic AMP may suppress the unregulated multiplication of transformed fibroblasts and impose contact inhibition of Chinese hamster ovary cells, it, and its inducers, are mitogenic for suspensions of rat thymocytes, causing these cells to proliferate.

PHOTOCHEMISTRY

Dark Photobiology?

from our Biological Chemistry Correspondent

THE susceptibility of the nucleic acids to photochemical change on ultraviolet irradiation has been thoroughly explored. The mutagenic and lethal effects which follow such chemical modification of the bases are, for the most part, relieved by enzyme repair systems. Even in those unfortunate individuals where such repair is defective, it has been generally assumed that the resulting photochemical damage would be solely restricted to those cells on the surface of the organism which are exposed to ultraviolet irradiation.

This restriction is now questioned by the elegant demonstration that pyrimidine dimers can be formed as a result of the generation of electronically excited molecules without light. Lamola selected trimethyl-1,2-dioxetane for this purpose because it is known to thermally decompose into excited state acetone and acetaldehyde and such carbonyl compounds have triplet-state energies closely matched to those of thymine and cytosine.

As expected, when the dioxetane was decomposed at 70° in the dark in the presence of *Escherichia coli* DNA, Lamola was able to isolate pyrimidine "photodimers" from both thymine and

cytosine residues (*Biochem. Biophys. Res. Commun.*, 43, 893; 1971). Although he estimates the efficiency of this process to be not much more than 1 per cent of the photosensitized reaction, the hunt must be on for other electronically excited promoters both to raise the level of dimerization and to provide a biological model.

There is no speculation in the photochemical modification of those tRNAs which contain 4-thiouracil because this base is excited by medium-wavelength ultraviolet light at 334 nm. Favre, Yaniv and their colleagues (*J. Mol. Biol.*, 58, 367 and 381; 1971) now provide some of the facts about the results of such irradiation. A 4-thiouracil at position-8 becomes covalently linked to a cytosine at position-13 in tRNA^{Val} and in five other tRNAs which all have those residues in common. Thus it seems likely that these six tRNAs have similar structures.

The nature of the linkage is not clear, though it is distinct from that in known photodimers and survives borohydride reduction of the tRNA in a modified form which provides a new fluorescent chromophore in the molecule. This fluorescence can be used to probe changes in the tertiary structure of the modified tRNAs and reveals that they have experienced very little change in segments of the tRNA other than that containing residues 8 and 13.

Contractile Microfilaments

A SURPRISING result has emerged from recent structural and chemical studies of motile processes in cells. The proteins actin and probably myosin as well, which were formerly thought to be present only in muscle cells, are in fact widely distributed in a variety of cell types. Microfilaments, structures 40–60 Å in diameter, which have been shown in some cases to be composed of an actin-like protein, appear to be involved in protoplasmic streaming, cytokinesis, formation of a ruffled membrane, nerve outgrowth and various morphological movements. In next Wednesday's *Nature New Biology*, Allison, Davies and de Petris describe the participation of microfilaments in phagocytosis and pinocytosis by mouse peritoneal macrophages.

Two techniques have been responsible for recent advances—an apparently specific test for the identification of actin-like microfilaments and a specific inhibitor of microfilament function. Heavy meromyosin forms a complex with actin in which the asymmetric myosin molecule is at 45° with the actin filament leading to a characteristic "chevron" appearance. Glycerina-

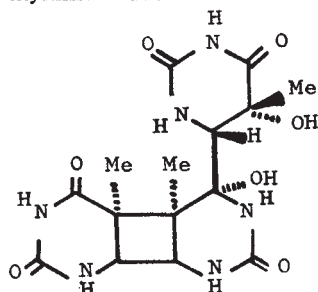
tion, which allows entry of the myosin, enables microfilaments to be identified within the cell. It was first shown by S. B. Carter (*Nature*, 213, 261; 1967) that the drug cytochalasin B breaks down microfilaments, and various functions of microfilament have been blocked by this compound (N. K. Wassells *et al.*, *Science*, 171, 135; 1971).

Allison and his collaborators show that macrophages contain bundles of filaments in the peripheral cytoplasm which bind heavy meromyosin. Cytochalasin reversibly blocks cell movement, ruffled membrane formation and pinocytosis in these cells. Phagocytosis of bacteria is also blocked even though the bacteria still attach to the plasma membrane. Treatment with colchicine, a specific inhibitor of microtubule dependent processes, does not effect pinocytosis.

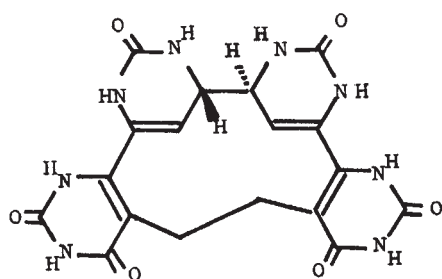
The ability to distinguish between the functions of microtubules and microfilaments by specific drugs and the isolation of both proteins have opened the way for an understanding of the functioning of fibrous proteins in intracellular and morphogenetic movements.

The thiouracil-cytosine linkage does, however, have small effects on the biological activity of the tRNA which is shown in a reduced affinity for the synthetase and a slower rate of incorporation of valine from the charged tRNA into polypeptides during protein synthesis.

The photoproducts so far isolated from DNA never contain more than two pyrimidines linked together. Wang, however, has isolated and characterized trimers and tetramers from irradiation of pyrimidines in ice matrices and in water (*J. Amer. Chem. Soc.*, **93**, 2554 and 2768; 1971) which could prove to have biological counterparts. The trimer in particular has the interesting property of reverting either to thymine and thymine dimer or to thymine and a thymine-adduct.



I



II

The structure (I) of the trimer and that of the tetramer (II), produced on irradiation of a thymine-uracil photoproduct, have been elucidated by Flippen and Karle (*J. Amer. Chem. Soc.*, **93**, 2556 and 2762; 1971) by X-ray diffraction.

ANTIBODIES

Garnering γ Globulin

from our Molecular Biology Correspondent
A 6 Å electron density map of an immunoglobulin has now been achieved by Davies and his colleagues (Sarma *et al.*, *J. Biol. Chem.*, **246**, 3753; 1971) and is in gratifying accord with what has been deduced about the shape of the molecule by electron microscopy and other methods. In the crystal the molecules have a dyad symmetry axis, and the electron density distribution is interpretable in terms of three globular

elements, joined together so as to form a T-shape, the stem consisting of the so-called Fc portion which is attached by means of a rather narrow hinge to the two Fab components. The hinge gives access to proteolytic enzymes, which cleave at this point, and is surmised from a variety of lines of evidence to be flexible when the molecule is in solution. The size and shape of the Fc unit are in good agreement with data derived from a study of the crystals of this fragment alone from Poljak's laboratory.

In a companion article, Lebow and Davies (*ibid.*, 3760) show electron micrographs of the immunoglobulin crystal. They utilize the magic of optical integration—whereby multiple prints of the micrographs are made, advancing the photographic paper each time under the enlarger by a distance corresponding to the periodicity. The picture of a well-dug field then gradually resolves itself into a sharply-defined pattern, in which the repeating unit stands out as a T or Y shape, somewhat smaller than the hydrated molecules observed by X-ray diffraction in the unfixed crystal,

but exactly like the isolated molecules observed in the electron microscope by Valentine and Green some years ago.

These γ G-immunoglobulin molecules consist of two heavy and two light chains, joined together by disulphide bonds, but other forms exist with a higher level of complexity. Thus electron microscopy has shown macroglobulin (immunoglobulin M) to consist of a cyclic pentamer of γ G-like (7S) subunits. The units are joined by disulphide bonds between their Fc regions. The determination of the sequence of events in the assembly, first of the four-chain structure, then of the pentameric form, has been one of the recent triumphs of the field, and some details of the mechanism are described in the latest article by Askonas and Parkhouse (*Biochem. J.*, **123**, 629; 1971). They showed previously that inside the tumour cells, in which the macroglobulin is made, only the 7S subunits are present. By the time the newly synthesized protein is secreted assembly to the complete pentamers has occurred. When the subunits from lysed cells are allowed to stand in air, no pentamers

The Truth about Juan de Fuca

THE appealing simplicity of seafloor spreading is probably an illusion on two counts. First, it would be surprising if a conceptual model so simple were to be rigorously applicable to a real Earth which is manifestly so complex, although the seafloor spreading model in its most basic form has done much to unify the Earth sciences. Current ideas on spreading will in the course of time no doubt come to be seen as a first approximation rather than as a detailed hypothesis for the behaviour of the Earth. But second, and perhaps more obviously, even if the seafloor is being, and has been, formed in a simple way, the final picture is likely to be quite complex. For notwithstanding the comparative youth of the oceans, changes may have taken place in the structure of the ocean floor since spreading began. Indeed, considering the state of activity in the oceanic crust it would be surprising if they had not.

As Peter and DeWald show in next Monday's *Nature Physical Science*, a good example of the latter type of complexity is the ocean floor off the north-west coast of the United States. Here the Juan de Fuca, Gorda and Explorer Ridges, each of which is offset from the others and each of which trends in a north-easterly direction, were clearly formed as a single rift but have since been subject to numerous fractures. The first job is thus to try to construct an authentic map of the anomalies and faults in the region; and this is what

Peter and DeWald have done, partly with the help of data previously published and partly with thirteen new east-west magnetic traverses carried out off the coasts of Washington and Oregon.

Examination of the pattern of magnetic offsets clearly shows the existence of a previously undiscovered fracture zone separating the mountainous ridge topography from the Tufts Abyssal Plain. This Peter and DeWald have termed, appropriately, the Juan de Fuca Fracture Zone. But, more importantly, the discovery of the new fracture taken in conjunction with the complete anomaly map has enabled Peter and DeWald to end the speculation about why the original ridge broke up and why its component parts now trend north-east. The fact is that some of the anomalies west of the Juan de Fuca Ridge are missing and obviously disappear along the newly discovered fracture zone which coincides with a 550-metre change in seafloor relief. This must indicate deformation involving rotation, strike-slip faulting and the over thrusting of small crustal plates; and according to Peter and DeWald the most likely cause is compression in the roughly north-east-south-west direction. What is now quite clear is that the ridge trends in this region cannot be attributable to anything as gradual as a slow realignment produced by a change in the direction of seafloor spreading.

are formed. The finished pentamers are easily reduced, and electrophoretic analysis shows that 7S monomers are first formed—as well, apparently, as some oligomeric intermediates. At higher concentrations of reducing agent the intra-subunit disulphide bonds begin to break, and HL pairs (one heavy with one light chain) appear, showing that the internal H-H disulphide bonds are next in order of lability. Finally, the remaining (H-L) links break and single heavy and light chains are produced. The 7S monomers, allowed to oxidize in air, regenerated the inter-subunit disulphide bonds and the pentamers were once more formed in high yield. When the free thiols were blocked with iodoacetamide no pentamers appeared.

Evidently then, the freshly synthesized, intracellular, subunits, which resist all attempts to make them polymerize, differ in the reactivity of their thiols, and Askonas and Parkhouse conjectured that these might be chemically protected. An obvious mechanism for easily reversible modification would be the formation of a mixed disulphide with a thiol present in the cell. Their surmise proved correct: treatment of the molecule with a reducing agent in low concentration converted it to a form that readily generated pentamers on standing. Exposure to the alkylating agent iodoacetamide before this reduction step had no detrimental effect, whereas alkylation after the reduction caused total inhibition of polymerization. The nature of the blocking group is unknown; cysteine or glutathione are obvious candidates, though an especially attractive alternative would be an intra-chain disulphide bond. Such a situation would be reminiscent of the formation of streptococcal protease from its zymogen by reduction of a disulphide bond. One must suppose that the liberation of thiols and polymerization occur during the passage of the immunoglobulin monomers through the membrane, where, it will be recalled, the disulphide-exchange enzyme is known to reside.

The earlier stage of synthesis—the association of light and heavy chains to give 7S subunits—has been studied by Parkhouse (*ibid.*, 635). The most stable disulphide bonds—those between light and heavy chains—are formed first, and the two HL fragments then associate. Pulse-labelling studies show that the light chains are present in a sizable pool.

Other disulphide-linked oligomers (IgA) are secreted by tumour cells of a different kind, and Parkhouse (*FEBS Lett.*, **16**, 71; 1971) has shown that here too only the 7S form is present in the cell. Oligomers up to the tetramer are formed; the disposition of the linking groups in this case evidently does not favour pentameric cyclization. In this

species, moreover, the association between heavy and light chains is non-covalent only.

HIGH ENERGY PHYSICS

The Elusive Quark

from a Correspondent

RECENT experimental studies at Livermore and Berkeley of more than 100,000 cosmic ray tracks from extensive air showers have failed to reveal any tracks made by fractionally charged particles and so another search for quarks has proved fruitless (A. F. Clark *et al.*, *Phys. Rev. Lett.*, **27**, 51; 1971).

The hunt for quarks has been going on since their existence was independently proposed by Murray Gell-Mann and George Zweig in 1964. They have been looked for since then with particle accelerators, in cosmic radiation, and in investigations of macroscopic material, for example seawater: most of these searches rely on the fact that the quarks should possess non-integral electric charges of one-third, or two-thirds of that of the electron, and should therefore yield tracks in particle detectors with substantially lower

ionizations than conventionally charged particles.

Although no substantiated evidence for the quark has been revealed by these investigations, there have been a few false hopes. In 1969, an Australian group, led by C. B. A. McCusker of the Cornell-Sydney University Astronomy Centre, claimed that certain anomalies, observed in cloud chamber studies of cosmic ray ionizations, were most likely to have been caused by quarks of charge $\frac{2}{3}e$ (*Phys. Rev. Lett.*, **23**, 658; 1969).

Five candidates for such quark events were reported from a total of some 6,000 cloud chamber photographs. This result was greeted with some scepticism by the physics community and was soon under severe attack: an article by R. K. Adair of Yale University and H. Kasha of Brookhaven (*Phys. Rev. Lett.*, **23**, 1355; 1970) indicated that the anomalously low ionizations reported by McCusker *et al.* were probably due to low energy electrons and muons associated with the air showers. A more recent report of a quark sighting by a group from Ohio State University, and based on a cosmic ray bubble chamber photograph, has also received very little support (W. T. Chu *et al.*, *Phys. Rev. Lett.*, **24**, 917; 1970).

Escaping Atmospheres

A CONTINUAL battle rages between the high-speed molecules trying to fly off from the Earth's atmosphere and the gravitational attraction trying to restrain them. For some planets, like the Earth and Venus, gravity has triumphed over levity up to now, but for the Moon, and probably Mercury, most of the escaping molecules have eluded their gravitational jailers; on Mars the issue is still in doubt, and a thin atmosphere persists, rather precariously.

The escape of molecules from the outer atmospheres of planets is determined chiefly by the temperature prevailing in the region, usually called the exosphere, where the mean free path of molecules is greater than the scale height—the height in which pressure and density decline by a factor of 2.8. In an article in next Monday's *Nature Physical Science*, S. J. Bauer describes a method for estimating this temperature in planets like Venus and Mars and hence the atmospheric escape rate.

If the molecules in the exosphere have thermal velocities higher than the escape velocity from the planet, the molecules travelling upwards are not very likely to suffer further collision, and will usually escape. The escape velocity for the Earth is between 10 and 11 km s⁻¹ and this is the thermal velocity corresponding to a temperature of about

4,000 K for hydrogen atoms (or 16,000 K for helium atoms). The actual temperatures at the base of the Earth's exosphere, at a height of about 500 km, are determined largely by the heating at lower heights (100 to 200 km), where solar extreme ultraviolet radiation is absorbed. On Earth it so happens that the solar radiation does not usually produce exospheric temperatures greater than 2,000 K, and even these temperatures are only maintained during a high maximum of solar activity, like that in 1957 and 1958. So, although there is no guarantee that the Sun will continue to be so cooperative, the present loss of hydrogen from the Earth's temperature is not too serious. There is always some loss, however, because the high-velocity tail of the Maxwellian distribution of thermal velocities can make its escape unchecked.

For the Earth, Bauer has calculated that the exospheric temperature at a strong solar maximum (2,000 K) is about 2.5 times that at solar minimum (800 K). For Venus and Mars, he suggests that the corresponding factor is about 1.7, and concludes that Earth and Venus have exospheres which, although subject to some loss, are stable even at solar maximum, whereas the atmosphere of Mars is stable only near solar minimum and suffers large losses at solar maximum.

The experimental technique used by Clark *et al.* was similar to that used by McCusker *et al.* and was based on cloud chambers triggered by coincidence counters. Artificially produced low-droplet count tracks were used to determine the detection efficiency for quark-like tracks, and from this the US group claim that their scanning team would have picked up four of the original five quark candidates found by the Sydney team. They concluded that no quarks had been observed, so that, for the present at least, quarks must remain essentially a convenient mathematical abstraction.

The story, however, is by no means over. On the theoretical side, the now fashionable parton picture of particle structure has stimulated renewed interest in quarks. Parton is the name given by theorist Richard Feynman of the California Institute of Technology to the unknown constituents of the nucleons and attempts have been made to equate the parton and the quark. On the experimental side, quark searches have been proposed for the giant new accelerator at Batavia, Illinois, and without a doubt the physicists working with CERN's new intersecting proton storage rings will maintain a sharp vigil for anomalously ionizing radiation. Meanwhile, most high energy physicists are still keeping an open mind on quarks, and awaiting further developments.

NARCOTICS

Antagonist Activities

from a Correspondent

FUNDAMENTAL to the symposium of the British Pharmacological Society on agonist and antagonist activities of narcotic analgesic drugs, held at the University of Aberdeen on July 12 and 13, was the concept that nearly all drugs of the group discussed possess both agonist and antagonist properties. This concept originated from the findings of L. Lasagna and H. K. Beecher in 1954 that the morphine antagonist nalorphine itself has analgesic properties, and of J. E. Villarreal about ten years later that some powerful analgesics of this group have enough antagonist activity to precipitate abstinence effects in morphine-dependent monkeys. The concept was quantified by H. W. Kosterlitz and his co-workers, who measured in the isolated ileum of the guinea-pig the opposed activities of drugs of this group, believed to correspond with their agonist and antagonist actions in the central nervous system. Using this simple model, these workers at the University of Aberdeen were able to place these drugs in descending order of the ratio of the agonist to antagonist activities that each possessed, making a

series beginning with the powerful analgesics heroin and morphine and ending with the seemingly "pure" antagonist naloxone. At the symposium, the validity of this procedure was supported by measurements of the antinociceptive and morphine-antagonist potencies of six drugs in this series in the rat and mouse by Drs H. O. J. Collier and C. Schneider (Miles Laboratories Limited, Stoke Poges). These measurements placed the drugs in an essentially similar order to that obtained with the guinea-pig ileum.

These experiments, both *in vitro* and *in vivo*, showed that the analgesic pentazocine possesses appreciable antagonist activity, placing it at a point some distance down the series from morphine in the direction of naloxone. That pentazocine is freer of addictive liability than is morphine or heroin supports the idea that this liability diminishes as the antagonist activity of an analgesic increases. A paper by Dr

G. F. Blane (Reckitt and Colman, Hull) and other contributions indicated that the advent of pentazocine has changed the search for new drugs of this group by lessening the acceptability of compounds with a high agonist to antagonist ratio and hence high addictive liability.

With the increase in drug addiction, much of the work reported at the symposium was slanted towards problems of dependence on opiates. The directions explored owed something to suggestions about the mechanism of drug dependence advanced during the 1960s by A. and D. B. Goldstein, W. D. M. Paton, H. O. J. Collier and others. Thus, Drs E. L. Way (University of California, San Francisco) and B. M. Cox (Chelsea College of Science and Technology, London) have found that drugs blocking protein synthesis inhibit the development of dependence as well as tolerance towards morphine. Again, Drs T. C. Muir and D. Pollock (University of Glasgow)

Agglutinins and the Cell Surface

DURING the past eighteen months plant agglutinins, or lectins as they are sometimes called, have become a major topic of conversation in many cancer research laboratories; for, as Burger and his colleagues at Princeton and Sach's group at Rehovot have shown, these substances preferentially agglutinate certain spontaneously and chemically transformed cells and some cells transformed by DNA tumour viruses. Moreover, a considerable collection of circumstantial evidence has been accumulated which suggests that the changes in the cell surface which can be monitored by measuring susceptibility to agglutination are intimately and perhaps causally connected with the regulation of cell division (see, for example, *Nature*, **228**, 502; and **228**, 512; 1970).

It has generally been assumed that, because transformed cells are more readily agglutinated by lectins such as concanavalin A and wheat germ agglutinin, transformation must result in the exposure of receptor sites which bind these molecules. In other words, the simple idea was that the transformed cell has more of these binding sites exposed on its surface than does the untransformed cell and that this surface change was somehow involved in the unregulated growth of transformed cells. In next Wednesday's *Nature New Biology*, however, the research groups of Ozanne and Sambrook and Cline and Livingston put a cat in this particular dove-cote.

Using either concanavalin A or wheat germ agglutinin labelled with ^{125}I or concanavalin A labelled with ^3H ,

they have shown that although BHK hamster cells and mouse 3T3 cells transformed respectively by polyoma virus and SV40 are more readily agglutinated than the corresponding untransformed cells, the untransformed and transformed cells bind, on a per milligram of protein basis, about the same amounts of both concanavalin A and wheat germ agglutinin.

In other words, there is no correlation between the amount of lectin a cell can bind and its susceptibility to agglutination by that lectin. The increased susceptibility to agglutination of transformed cells does not result from the exposure of more lectin-binding sites at the cell surface. The most convincing and elegant proof of these statements is provided by Ozanne and Sambrook who have measured the binding capacity and agglutinability of hamster BHK cells transformed by the temperature sensitive mutant of polyoma virus *ts3*. Growing at 32° C these cells are agglutinated by low concentrations of concanavalin A or wheat germ agglutinin but when they are shifted to 38.5° C much larger amounts of the lectins are needed for agglutination. The amount of lectin bound, however, is the same at both temperatures.

Clearly, increased susceptibility to agglutination reflects surface changes more subtle than an increase in the number of lectin-binding sites and as Ozanne and Sambrook say "it seems difficult to postulate a general mechanism of growth control based solely on the number of exposed lectin binding sites".

reported that, in the rat, the response of smooth muscles to adrenergic or cholinergic stimulation greatly increased after the withdrawal of chronic treatment with morphine—reaching a peak three days later.

In another direction, the mechanisms of dependence suggested some years ago require an interaction of morphine with a humoral mediator involved in the activity of neurones in the brain. In this direction, some setbacks and some encouraging findings were recorded. On the one hand, the evidence adduced by Drs A. Goldstein (Stanford University) and I. Marshall (St Mary's Hospital Medical School, London) was against the proposition, stoutly defended by Dr Way, that a change in the level or turnover of brain 5-hydroxytryptamine is important in morphine dependence in the mouse. On the other hand, C. B. Smith and J. E. Villarreal (University of Michigan, Ann Arbor) reported that continued treatment with morphine and other addictive opiates increases the incorporation of ^{14}C -tyrosine into the noradrenaline and dopamine of the brain of the mouse. The increased turnover of brain catecholamines can not be induced by strong antagonists of morphine and is blocked by naloxone; in the author's opinion it is correlated with addictive liability in this group of drugs.

BACTERIOLOGY

More Pollution

from a Correspondent

THE summer conference of the Society for Applied Bacteriology was held at the University of Liverpool from July 13 to 15 and took the form of a symposium on the microbial aspects of pollution. The contributions and subsequent discussions interpreted the title in the broadest possible terms so that well discussed topics, such as the pollution of the aquatic environment, and lesser known forms of pollution, such as the disposal of infected laboratory materials, were included. Few new data were presented, but they illustrated the importance of microbiological processes in the armoury for combating today's pollution problems.

Dr A. L. Downing (Water Pollution Research Laboratory, Stevenage) warned microbiologists not to get too excited at the prospect of an increased number of jobs for their profession in this expanding field because the introduction of new and more stringent existing standards for effluent quality could well result in non-biological methods becoming increasingly more important.

Problems pertaining to the eutrophication of surface waters were covered

in detail by four speakers. Professor P. L. McCarty and Dr R. T. Haug (Stanford University, California) discussed the various methods available for the removal of nitrogen from sewage effluents by the microbiological processes of nitrification and denitrification and presented evidence for supporting the use of aerobic and anaerobic upward-flow submerged filters. Dr N. P. Burman (Metropolitan Water Board, London) pointed out, however, that the removal of nitrogen and phosphorus from sewage effluents was not always the answer to eutrophication problems and gave the example of the Thames valley reservoirs where eutrophic conditions were inevitable because sufficient quantities of these elements were available from sources other than sewage.

Aerobic and anaerobic biological treatment of sewage and sewage sludges was discussed from both the microbiological and applied points of view and it was particularly encouraging to hear that the lead given by the deter-

gents industry which changed from the production of biologically "hard" to "soft" materials is seriously being considered by industries producing other widely used materials. First, Dr H. O. W. Egging (University of Aston) spoke on the possibilities of the plastics industry producing materials with parts in the chemical chain susceptible to microbial attack which would cause depolymerization and allow the subsequent attack of the smaller molecules by other microbes. Second, Dr S. L. J. Wright (Bath University of Technology) stated that a particularly attractive idea concerning the degradation of herbicides was the possibility of spraying the treated soil with an appropriate active microbial culture after an optimum time; preliminary experiments had already met with success. Third, Dr R. E. Cripps (Shell Research Ltd, Sittingbourne) spoke of his work on the relation between the chemical structure of pesticides and the possibilities of their degradation by microbial populations.

Chromosome Replication in Two Directions

FOR the double stranded and covalently closed circular DNA molecule which constitutes the chromosome of *Escherichia coli* to replicate in a semiconservative fashion the two parental strands of DNA must separate. There is, of course, evidence indicating that this separation is achieved by breaking phosphodiester bonds of the backbone of one of the two strands such that the nicked strand can be unwound from the circular strand. (Whether a single nick suffices or the chromosome is repeatedly nicked during replication is in the long run immaterial, for the net result is the same.) Moreover, genetic experiments have shown that replication begins and proceeds sequentially from a fixed point on the chromosome, the origin of replication. In theory at least replication might proceed in one or in both directions from this origin and until now it has generally been assumed that replication is unidirectional, proceeding 360° from the origin back to it. In next Wednesday's *Nature New Biology*, however, Masters and Broda challenge this notion; their experiments lead to the conclusion that replication occurs in both directions from the origin.

Masters and Broda have, in essence, measured the frequency of occurrence of particular genetic markers situated at either side of the origin of replication in cultures of cells growing at different rates. They used the generalized transducing phage P1 which picks up *E. coli* genes at particular frequencies dependent on the number of copies of

the genes present in the cell and the ease with which they can be integrated into the phage genome.

Ratios of the frequency of particular genetic markers measured in this way fall on a two directional gradient when plotted against the markers' genetic map positions. Moreover, a series of elegant transduction experiments with various male and female strains of *E. coli* indicate that the relative frequency of transduction of a particular marker depends on the position of that marker in the chromosome.

In dividing cells it seems that there are more copies of markers immediately adjacent to both sides of the origin of replication than there are copies of genes distal from the origin and genes close to the origin are more often transduced than genes distal from it. Both these findings indicate that replication proceeds in both directions from a fixed origin. Furthermore, assuming that the current genetic map accurately represents the length of DNA between markers, it seems that the rate of replication in the two directions is not the same. The terminus of replication is not 180° from the origin and replication in a clockwise direction about the chromosome seems to be faster than replication in the counter-clockwise direction. In other words, Masters and Broda claim that replication of the *E. coli* chromosome is bidirectional but asymmetric and, as they note, Caro and his colleagues have reached the same conclusion from quite different experiments.

Why Hydrology?

JOHN C. RODDA

Institute of Hydrology, Wallingford, Berkshire

The International Hydrological Decade is now more than six years old and decisions about future international cooperation must soon be taken if the impetus given to hydrology during this period is not to be lost.

WHY hydrology? What is the point of studying the circulation and distribution of the world's freshwaters? Why bother with this science concerned with the rain and what happens to it when, in Britain at least, there always seems to be so much of it? Perhaps these questions and others like them were relevant twenty or thirty years ago, but not today; not when the consumption of water in the United Kingdom is rising at such a rate that demand will probably double by the end of the century and when the dry weather flow in a large number of rivers consists almost entirely of treated effluent from sewage works. In fact the Central Advisory Water Committee has recently made it clear¹ that re-use of this treated water will become increasingly important for supply purposes in the future.

Then at the other extreme, there are the floods that affect different parts of the country each year and can cause damage costing several million pounds annually. Fortunately loss of life occurs less frequently, but there can always be other disasters on the scale of that at Lynmouth in 1952. In fact there is some indication that the incidence of heavy rain is increasing; for example, on July 10, 1968, parts of Somerset and Gloucestershire recorded more than 130 mm of rain in 18 h, and in September of the same year parts of Kent, Surrey and Sussex experienced rainfalls of similar intensities. Even these rates of rainfall are, however, low on a world scale that reaches 1,500 mm a day for some Indian Ocean islands. One consequence of these relatively low intensities is that soil erosion is not the problem in Britain that it is, for example, in New Zealand. Nevertheless rivers draining the moorlands and mountains transport considerable amounts of sediment during flood, particularly when hill pastures have been ploughed recently. This also applies to almost any part of the country where building and construction are in progress on a large scale.

Facing the Hazards

Flood, erosion, drought and pollution are high on the list of hazards confronting man and they are also the chief problems facing the hydrologist. Can floods be forecast and drought severity predicted? Can the controls of the quality of the water flowing in a river be understood satisfactorily? These are questions that involve hydrologists the world over, but the fact that vast sums of money are spent in water supply schemes, flood alleviation works and sewage treatment plants, in order to mitigate the effects of these hydrological hazards, is usually taken for granted. In the same way, minimal attention is paid to the continuing research programmes into water problems which provide a basis for the design of these schemes and works. The aims and objects of the International Hydrological Decade (IHD), for example, are relatively little known.

Other worldwide scientific programmes such as the International Geophysical Year (IGY) and International Biological Programme (IBP) have received far wider publicity, but perhaps none of these is as basic to the needs of modern civilization. The objectives of the IHD are to foster international collaboration and cooperation in hydrology and to take stock of the world's freshwater resources, to increase the effectiveness of research into such problems as floods and the effect of man on the hydrological cycle, and to improve the knowledge needed for the future management of water resources.

The IHD started in 1965 and was the outcome of an idea conceived in the early 1960s. At a preliminary meeting, representatives of fifty-seven nations agreed that their governments should support the United Nations Educational Scientific and Cultural Organization (UNESCO) in launching an international cooperative programme in hydrology; now the IHD involves more than 100 countries as well as other international organizations such as the Food and Agriculture Organization of the United Nations (FAO) and the World Meteorological Organization (WMO). More than fifty technical items appeared in the original programme, and these included improving methods of measuring the basic factors of precipitation, evaporation and river discharge and making more effective use of maps for hydrological purposes. Proposals were made for standardizing methods of observation, for training and educating hydrologists in developing countries and for giving support to symposia on important topics. Progress up to 1969 was reviewed at the Mid-decade Conference, which was held in Paris in the December of that year, and both the programme for the remaining five years and plans for hydrology after the conclusion of the decade were also discussed. This question is now being considered by a special working group set up to make proposals for a long term UNESCO based programme in scientific hydrology. One problem is how to devise a means of fostering hydrology internationally, which is not just a revamping of IHD ideas spread over a longer time; another is that developing countries have special needs that may not be catered for in an intensely scientific programme. There are also the roles of the other UN agencies to be considered and the position of the International Association of Scientific Hydrology (ASH), one of the members of the International Union of Geodesy and Geophysics (IUGG). The FAO, for example, has its special interest in applying hydrology to the betterment of agriculture and the WMO is embarking on its own programme for operational hydrology. What is more, the meeting of the World Meteorological Congress in April 1971 gave consideration to the Organization's future role in hydrology and discussed to what extent it should cater for the needs of hydrological services in addition to those of the meteorological services. The question really is whether the WMO should become the World Hydrometeorological Organization at some future date. There are hydrometeorological services in a number of countries such as Sweden and the USSR, and these could be the basis of an obvious extension for WMO. Another possibility would be to found a new UN agency with interests spanning the entire field of hydrology and water resources. This would avoid the apparent "takeover" of hydrology by an allied discipline or the alternative—that it becomes an insignificant part of the programme of a large UN agency. On the other hand, there is the important question of finance and the fact that UNESCO, WMO and FAO and

possibly WHO, the International Atomic Energy Agency (IAEA) and the UN Water Resources Development Centre would find it exceedingly difficult to divorce themselves from their present responsibilities in these fields.

Divided Responsibilities

It may seem rather odd that these international complexities have a parallel in Britain, but here too the responsibilities for water are divided. Depending on whether the fluid is considered a commodity, a danger, as a means of transport or as a subject demanding attention from the research worker, different bodies are involved. Water undertakings, sewage disposal authorities, government departments, the Central Electricity Generating Board and British Waterways have their own, sometimes competing, interests, to say nothing of industry, agriculture and those who think of water for sport, recreation and as an amenity. As a rule the unit of operation is small. In England and Wales there are more than 200 water supply undertakings, 1,300 sewage disposal authorities and 26 river authorities. As far as research is concerned there are 20 or more government bodies involved, as well as the universities. In fact the recent Natural Environment Research Council (NERC) Survey of Hydrological Research in the United Kingdom² revealed a tremendous wealth of research, much of it long term, being undertaken in a wide variety of establishments. One suggestion is that the most substantial parts of this research should, in future, come under the aegis of a new Central Water Authority³; but in view of NERC's existing statutory responsibilities and present commitments for research in hydrology, perhaps a strengthening of these responsibilities and better coordination centred on NERC would be a more appropriate solution.

Of course, it could be argued that the organization of hydrology, whether nationally or on a global basis, has little relevance to the drought stricken Indian village or the wide areas which the Danube has flooded. Yet the sharing of a river basin with other nations is in itself a compelling reason for international cooperation. Hence there are commissions or some form of control body for a number of the world's principal rivers, the countries concerned often being assisted by one or more UN agencies. Then there is the need to standardize instruments, methods of observation, methods of data processing and analysis so that hydrological information knows no frontiers. For example, there are considerable differences in the instruments used in various countries for the measurement of rainfall and evaporation—two basic elements in the water balance. To produce a rainfall map of Europe is an extremely difficult task, the most important reason being that each country uses its own type of gauge, which often differs radically in size, shape, material and method of installation from those used by its neighbours.

Evaporation is even more of a problem, for few countries have widespread networks of evaporation pans or tanks and some have none of these instruments at all. Not many have the detailed records of climate that are needed for determining evaporation indirectly, by the Penman method for example. There is a comparison of evaporation pans and tanks sponsored by WMO in progress at the moment. A number of meteorological stations around the world, for example Kew Observatory, have been equipped with US National Weather Service Class A pans, the GGI 3000 and 20 m² pans of the USSR Hydrometeorological Service and several other types of instruments; a number of years of data has now been amassed. A similar but unsatisfactory series of comparisons of rainfall gauges has been based on the Interim Reference Precipitation Gauge; unfortunately this type of instrument, a 1 m high gauge fitted with a wind shield, was as susceptible to aerodynamic effects as any other type of elevated gauge—these effects cause acceleration of air moving over the top of the gauge and consequently a diversion of drop trajectories away from the orifice. This problem can be almost completely overcome by installing a

gauge in a shallow pit so that its rim is level with the ground surface and surrounding the gauge with an anti-splash grid. Proposals for a project based on this type of gauge were made at a WMO meeting at the Institute of Hydrology in July 1970 and the comparison of national rain gauges with these instruments commenced in June this year. Tests of these ground level gauges have been carried out in some countries already. In the USSR, comparisons of national standard gauges and ground level gauges made at a large number of sites have resulted in the re-drawing of precipitation maps and an upward scaling of values. Results from a number of sites in the United Kingdom indicate that the existing 1 foot high standard gauge network measures less rain than actually reaches the ground. Annual rainfall totals are between 3 and 7% greater than those recorded over most of south-east England, while the deficit can be up to 20% for unfavourable sites in the north and west.

Basin Programme

One of the most successful aspects of the IHD has been the representative and experimental basin programme which has aided the improvement of basic countrywide hydrological networks in less well developed countries and has been a stimulus to research in the more developed ones: these studies of complete catchments and their water budgets highlight the need for specialized instruments and improved methods of data processing and analysis. Several countries have planned their networks of representative and experimental basins; the one designed to sample the principal hydrological regions and the other to sample the chief changes in the use of land. In others, Britain for example, the growth of these catchment studies has been rather haphazard. A survey of basin studies undertaken by NERC in 1968 showed that there were almost fifty such projects in progress within the United Kingdom. Certain parts of the country and certain objectives were adequately covered, for example studies of the effects of afforestation in upland Britain. On the other hand, there were only two studies being undertaken in lowland England on the consequences of urbanization—one of the most radical of all land use changes. The hydrological significance of snow, particularly in the Scottish Highlands, was also not being investigated—quite a serious gap which still remains.

The situation in Australia is rather different. About eighty physical regions have been delimited using indices of geomorphology, geology and rainfall, and a representative basin is being established in each⁴. Various government organizations and universities are participating in these activities which are being promoted by the Department of National Development. Records from each basin are being assembled in computer compatible form and will be made available to the CSIRO Division of Land Research, Canberra, for analysis. For the analysis, a rainfall-runoff model has been devised⁵ and tested with data from a basin near Alice Springs. Predictions of storm runoff are produced from observations of rainfall, potential evaporation and a number of other factors.

The New Zealand network of representative basins has also been planned rationally⁶. The two islands have been divided into about ninety hydrological regions, a representative basin being established in nearly all of them by the Water and Soil Division of the Ministry of Works. These catchments sample the principal precipitation zones, the chief soil types and the important geomorphological areas; they range in size from 1 to 250 km². There are also a number of experimental basins in operation such as those at Moutere near Nelson, where the significance of various changes in land use is being studied in twelve small catchments⁷. One experiment at this site is concerned with cultivating a gorse catchment and studying the resulting change in the water budget and the differences in the distribution of stream discharge. Peak flows were found to increase, annual runoff totals were nearly doubled, but there were fewer days when runoff occurred. This and similar studies are particularly important for New Zealand, where immense

Fig. 1 Trapezoidal flume used by the Institute of Hydrology for gauging flows from the forested headwater catchment of the River Severn in the Plynllyon experimental catchment where the hydrology of forest and upland sheep pasture is being compared.



problems have resulted from imported land use practices and their effects on soils and water.

Measuring Advancement

The nature of these problems and the strenuous efforts being made to find solutions to them may have been one of the reasons for holding in December 1970 the symposium on the results of research on representative and experimental basins at Wellington, New Zealand. This symposium, organized by the Royal Society of New Zealand and arranged by the International Association of Scientific Hydrology with the support of UNESCO, was attended by over 150 hydrologists from more than thirty countries. Earlier in the year, the World Water Balance symposium had been held at Reading University and had attracted more than 250 participants from about fifty countries. After the Symposium about sixty of the participants went on a tour of Central Wales and the Midlands. One of the sites visited was the Institute of Hydrology's Plynllyon Catchments, where a long term study of the contrasting headwater basins of the Wye and Severn was commenced in 1967. The basin of the Wye is almost entirely sheep pasture, while the adjacent Severn is nearly all planted softwood forest—an ideal situation for investigating the hydrological consequences of these land use differences. For large areas of upland Britain are being afforested, but the effects of this change in land area river flows are not clear. Are floods reduced in severity? Do flows diminish more rapidly during dry periods? By measuring rainfall, evaporation, and changes in soil water storage, in addition to river flows, it is anticipated that the magnitude of any alterations to the hydrological regimes of these areas can be established, as well as the causes. Data are now being collected from specially constructed river gauging stations in the two basins (Fig. 1) and from fifty rain gauges and fifty soil moisture sites distributed over the catchments. Five automatic weather stations will be installed shortly for assessment of evaporation to supplement the two conventional stations that have been operating for some time. Initial results show that there are substantial differences between the two areas that must result from the contrasting land usage. Both the Reading and Wellington meetings took place within the framework of

the IHD and they provided valuable forums for the exchange of ideas and the promotion of contacts between individuals. Indeed it has been said that the IHD has been better for hydrologists than for hydrology, but it is difficult to see how the benefits can be separated.

Measurement of the advancement of a science is not, of course, an easy task; progress is never continuous but rather a series of breakthroughs and periods of consolidation. Sherman's inceptive unit hydrograph paper⁸ was the breakthrough in the 1930s, followed by Gumbel's work⁹ on extreme value theory and Penman's studies of evaporation¹⁰ a decade later. But similar progress after 1950 is difficult to pinpoint in the same way. Perhaps the computer revolution and particularly the spate of mathematical models of catchment behaviour that has resulted is one of the most noteworthy features. Advances in methods of analysis have certainly been rapid recently, so much so that faults in the basic measurements are becoming one of the chief hindrances to further progress. Britain seems to be well placed, for there are few countries with equal experience in instrumentation, particularly in rainfall measurement, stream gauging and soil moisture assessment. It could even be argued that the problems resulting from Britain's relatively recent start in scientific hydrology are rapidly being overcome. Hydrology is now an established science, a science that is extremely valuable to the community at large, though not one that captures the interest it deserves.

¹ *The Future Management of Water in England and Wales* (Central Advisory Water Committee, HMSO, 1971).

² *Hydrological Research in the United Kingdom (1965–1970)* (Natural Environment Research Council, 1970).

³ *Seventh Annual Report* (Water Resources Board, HMSO, 1971).

⁴ *The Representative Basin Concept in Australia*, Hydrological Series No. 2 (Department of National Development, Canberra, 1969).

⁵ Chapman, T. G., *Proc. IASH Symposium on the Results of Research on Representative and Experimental Basins, Wellington*, 126 (1970).

⁶ Toebes, C., *Proc. IASH Symp. Budapest*, 147 (1965).

⁷ Scarf, F., *J. Hydrol. (New Zealand)*, *Proc. IASH Symp. of Wellington*, 9, No. 2, 142 (1970).

⁸ Sherman, L. K., *Engineering-News-Record*, 108, 501 (1932).

⁹ Gumbel, E. J., *Amer. Geophys. Union Trans.*, Part III, 836 (1941).

¹⁰ Penman, H. L., *Proc. Roy Soc., A*, 193, 120 (1948).

Prospects for Carbon Fibres

R. JEFFRIES

The Cotton Silk and Man-made Fibres Research Association, Shirley Institute, Manchester

The relatively high price of composites reinforced with carbon fibres still means that they will only be used when their strength, stiffness and lightness are absolutely essential. But in the future, industries as diverse as the chemical industry and shipbuilding could reap the benefits of a cheaper product.

CARBON fibres made from cellulose fibres have been known for a considerable time. They were not sufficiently strong or stiff to be of any real value for the reinforcement of composite materials and were manufactured for their thermal insulation and ablative properties, and for use in filter beds and so on. A team at the Royal Aircraft Establishment (RAE) first made carbon fibres with tensile properties good enough to warrant the term "high performance" in relation to reinforcement^{1,2} and at about the same time, at the Aero Engine Division of the former Rolls-Royce Ltd, similar techniques were also being developed for producing these high performance carbon fibres; rapid progress was made in translating this laboratory work into engineering practice. The RAE and Rolls-Royce carbon fibres are produced by the controlled thermal degradation of a special grade of acrylic fibre made by Courtaulds Ltd (for example refs. 2-5). In the three-stage heating process (for example refs. 2-7) the fibre is first oxidized by heating in air at 200° to 300° C; it is then carbonized by heating in an inert atmosphere to about 1,000° C and finally the carbonized fibre is graphitized to a greater or lesser extent by heating (also in an inert atmosphere) to a temperature in the region 1,500° to 3,000° C. Carbonization removes almost all nitrogen and hydrogen from the acrylic fibre and a proportion of the carbon, leaving a fibre made substantially from carbon—hydrogen cyanide and ammonia are the chief gases evolved⁸⁻¹³. Graphitization "crystallizes" the carbon fibre to some extent, that is it causes the carbon structure to organize itself into a more regular arrangement similar to (though not the same as) natural graphite. The mechanical properties of the final fibre depend on the temperature of the graphitization stage of the preparation process and it is therefore possible by this means to produce different types of carbon fibre.

An important feature of the process is the maintenance of the high orientation in the acrylic precursor throughout the carbonization processes^{2,3,5}; to this end, tension is applied to the fibre during the early stages of the process to prevent any shrinkage and structural disorientation. It is partly the use of tension which distinguishes the process of Watt and his co-workers from that of Shindo⁸ who also studied the preparation of carbon fibre from an acrylic precursor.

Each stage of the heating programme has presented considerable development problems, particularly in turning the process into a large scale operation; for example, the oxidation stage is exothermic in character and liable to get out of control⁶.

These problems were solved, however, and production of the

fibre in commercial quantities has been under way for some time. Two forms of process have been developed: in the first the material is prepared by a batch process, in which carbon fibres are produced with lengths of about a metre. A continuous process has more recently been developed which yields material with lengths up to about 1,500 m.

Properties and Performance

High performance carbon fibres are black, lustrous, rather slippery to handle and are at present produced with diameters of 8 to 9 μm (ref. 3). Their mechanical properties are outstandingly good (Table 1, from refs. 1, 14-17) and values quoted by other manufacturers of carbon fibre (Rolls-Royce, Courtaulds, and Morganite) are all similar in magnitude. Each value of strength and modulus in Table 1 is divided by specific gravity to give the specific strength and modulus; these specific values should always be considered when comparing materials on a weight for weight basis. The carbon fibres (both the high strength and high modulus types) are far superior to the steel. The glass fibres (particularly the S type) are similar in strength to the carbon fibres but have a much lower modulus. It is this very large stiffness that makes carbon fibre such an important reinforcing material for engineering applications; high strength needs to be associated with high modulus (that is, high stresses have to be borne at low extensions) in most engineering materials. The carbon fibres show completely elastic behaviour up to the breaking point and so there is no irreversible extension or creep. The fibres are comparatively weak and of low modulus in the direction perpendicular to the fibre axis; for instance, the Young's modulus of graphite at right angles to the graphite planes is only about 5×10^6 pound inch⁻² (ref. 18).

As the temperature of the third stage of the heat treatment¹⁶ is increased in the range of 1,000 to 2,500° C the modulus steadily increases and the tensile strength at first increases, reaches a maximum at about 1,500° C, and then decreases. It is thus possible to prepare a high modulus fibre, at the expense of some strength, or a high strength fibre at the expense of some modulus. The difference between the "high modulus" and "high strength" fibres in Table 1 arises from the differences in the temperature of the final heat treatment. Courtaulds and Morganite manufacture both high modulus and high strength fibres; Courtaulds type HT and Morganite type II are high strength, Courtaulds type HM and Morganite type I are high modulus. Courtaulds also make a Type A fibre which is of somewhat lower strength and modulus than the type HT, but has certain other advantages that will become clear later³.

The standard deviation of the strength values of carbon fibres is about 25% (refs. 1 and 14); this is because of structural flaws which cause premature breakage. By comparison, the Young's modulus of the fibre, being an intrinsic property of the fibre and therefore not influenced by flaws, is much less variable and the standard deviation is only about 5% of the mean. Flaws of a discrete, "macroscopic" nature may, however, be the strength-controlling factor only for carbon fibres prepared at temperatures up to 1,300° C (refs. 19 and 20); at higher treatment temperatures some other strength-controlling mechanism seems to take over. It has been suggested²¹ that the

strength of the fibres may be governed in some circumstances by microcracks formed during cooling from the processing temperature.

Carbon fibres are very resistant to heat in inert conditions and the volatilization temperature is more than 3,000°C; they begin to oxidize in air at 410 to 450°C, however. The thermal and electrical conductivities along the length of the fibre are lower than the values for a heat treated pyrolytic graphite in the direction parallel to the planes in the graphite structure²², showing the effect of the small crystallite size in the fibres.

Fibre Structure

The structure of carbon fibres is similar to that of graphite—the atoms are in layers or planes and the planes are arranged almost parallel to each other. In true graphite, however, the atoms in each plane are arranged in a regular manner with respect to those in the adjacent planes, but in carbon fibres there is no such regular arrangement and the structure is described as turbostratic²².

The planes of atoms are well oriented in the direction of the fibre axis¹—in the case of a high modulus fibre to within 10° of the axis. This high degree of orientation is the reason for the high modulus of the fibres¹, and any treatment that increases the orientation invariably increases the modulus, regardless of whether the increased orientation is imparted by stretching the precursor acrylic fibre^{23,24}, by the application of tension during

composites^{34–36}. In the “leaking mould” technique, the fibre is combined in the mould with a solventless liquid polyester or epoxy resin and then pressed; the excess resin is forced out of the leaky mould. In the “prepreg” method, layers of fibre are impregnated with heat-setting resin in dilute solution and dried. These preimpregnated layers are then moulded together in the normal way under heat and pressure, and cured. Filament winding techniques involve the building up of layers of fibre (intermixed with resin) by winding the fibre onto a rotating former; more or less continuous lengths of fibrous material are obviously needed for this technique. Random composites are made by mixing chopped carbon fibre with resin and subjecting the mixture to the usual moulding techniques.

The mechanical properties of the carbon fibre-resin composite depend on the mechanical properties of the fibre, the volume fraction of the fibre in the composite, the orientation of the fibre in the composite, and the strength of the fibre-resin bond. Phillips³⁵ has described the various properties of unidirectional composites (those containing fibres oriented in only one direction) made with RAE high modulus fibre and a polyester resin at a volume fraction of fibre of about 40% (50% by weight). The properties in the direction of fibre orientation, as listed by Phillips³⁵, are shown in Table 2. In spite of the diluting effect of the resin, the specific strength and modulus values of the composite in the direction of fibre orientation are still much superior to that of any of the three

Table 1 Comparison of Properties of High Performance Carbon Fibres with Other Materials

Property	RAE carbon fibre High modulus	High strength	Steel wire	S-glass fibre	E-glass fibre	Bulk steel	Bulk aluminum	Bulk titanium	Cotton	Textile viscose	Polyester fibre
Tensile strength (10 ³ pound inch ⁻²)	300	430	400–600	650	500	145	67	135	70	40	90
Extension at break (%)	0.5	1.3	~2	~5	~5				9	20	22
Young's modulus (10 ⁶ pound inch ⁻²)	60	33	30	12.5	9	30	10.5	16	0.6	1–2	1.8
Specific gravity	2.0	1.74	7.8	2.5	2.5	7.8	2.8	4.5	1.55	1.51	1.38
Specific tensile strength	150	250	51–77	255	200	18.5	24	30	45	26	65
Specific Young's modulus	30	19	3.8	4.9	3.5	3.8	3.75	3.5	0.4	0.65–1.3	1.3

the oxidation stage^{25,26}, or by stretching the final carbon fibre during the graphitization process^{27–29}.

The carbon fibres are composed of aggregates of crystalline regions separated by grain boundaries and pores. These crystallites in graphitized fibre are thought to be about 40 to 100 Å wide^{21,22,30,31} and about 60 to 120 Å long in the fibre direction^{21,22,30}; the dimensions are somewhat less in ungraphitized carbon fibre^{22,31,32}. This polycrystalline nature has already been mentioned in connexion with the electrical and thermal conductivities of the fibres.

The specific gravity of RAE carbon fibres (Table 1) is much lower than that of true graphite, which is about 2.26. This may be partly the result of imperfect graphitization but is more likely to reflect the presence of a pore structure in the fibre. Johnson and Tyson³⁰ consider that the voids separate the short crystals in the graphitized material, and have a mean width less than 10 Å.

There is evidence^{21,22,33} that RAE carbon fibres have some fibrillar character and the suggestion has been made that the strength of the fibre is a function of the interfibrillar bonding. Evidence has recently been adduced that sheet-like structures are also present in carbon fibres³³.

Composites

If the exceptional properties of carbon fibres are to be utilized for engineering purposes they must be fabricated as a “reinforced plastic”; the fibres are aligned in the required direction or directions and embedded in a suitable resin. There are three types of technique for preparing “directional”

bulk metals in Table 1. The modulus and flexural strength of the composite are almost unaffected by immersion in cold or boiling water³⁷—the carbon fibre-resin interface is weakened by less than 5%, whereas the interface between glass fibre and resin is weakened by as much as 15%. The fatigue life of the composite is very good, and the damping properties are also good. The properties of carbon fibre reinforced plastics, made with Courtaulds and Morganite carbon fibres, and also with carbon fibres produced in the United States have been summarized by Dauksys and Ray³⁸.

The properties of carbon fibre reinforced plastics depend to some extent on the strength of the bond between the fibre and the resin. Too weak a bond leads to a low interlaminar shear strength (ILSS) whereas too strong a bond causes the

Table 2 Properties of Carbon Fibre-Resin Composites

Property	Unidirectional carbon fibre-resin composite ³⁵	Bulk steel	Bulk aluminum	Bulk titanium
Tensile strength (10 ³ pound inch ⁻²)	105	145	67	135
Young's modulus (10 ⁶ pound inch ⁻²)	22	30	10.5	16
Specific gravity	1.54	7.8	2.8	4.5
Specific tensile strength	68	18.5	24	30
Specific modulus	14	3.8	3.75	3.5
Fatigue resistance in fluctuating tension (50,000 ± 20,000 pound inch ⁻² stress level)	> 20 × 10 ⁶ cycles to failure			

material to be brittle; a compromise has therefore to be reached^{38,39}. The ILSS of carbon fibre reinforced plastic made with untreated carbon fibre (particularly high modulus fibre) is lower than is desirable for many purposes but both Courtaulds and Morganite now market both high modulus and high strength fibres that have been specially treated to improve the fibre-resin bond and thus increase the ILSS. Courtaulds also market a carbon fibre which, although somewhat less strong and stiff than the other two types, produces composites of satisfactory ILSS even without treatment; it is cheaper than the other types, and has a breaking strain of more than 1% which is advantageous for some purposes. An alternative means of increasing the ILSS is to whisker the carbon fibres, that is, to cover the fibre with large numbers of randomly oriented, short whiskers^{40,41}. Giltrow and Lancaster⁴²⁻⁴⁴ have shown that the presence of carbon fibre in a resin reduces the coefficient of friction and rate of wear.

Fig. 1 Photomicrograph of a longitudinal section of a carbon fibre resin unidirectional composite tested to failure.

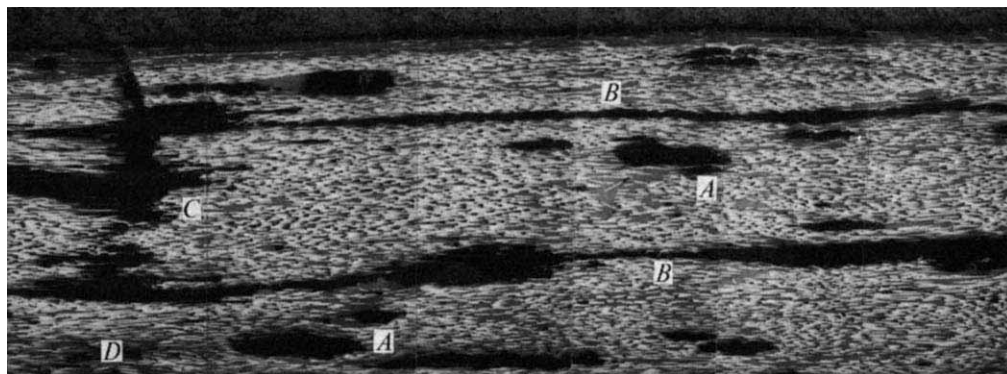


Fig. 1 shows a photomicrograph (at a magnification of about 40) of a longitudinal section of a carbon fibre resin unidirectional composite tested to failure in a three-point short-beam bending test; the composite contains 60% of fibre in an epoxy resin. The photomicrograph illustrates the various faults that can be present in this type of composite as manufactured (this is, in fact, a poor specimen of composite chosen to reveal these various faults), and also the additional features which appear as a result of stressing the composite to failure in this type of test. In the photograph, the fibres show up white, the resin grey, and voids and cracks appear black. The three types of fault present in untested specimens are voids (marked *A* in the photograph), places where the concentration of fibre is very low, and misaligned fibres. Each of these faults are potential weak points in the composite, particularly the voids; it is likely that, in the case of this specimen, the failure mechanism originated at voids. When a composite is tested in the three-point test, the specimen first fails in shear, and the long cracks (*B*) are the result of this type of failure. Further stressing of the specimen leads to compressive failure, characterized by the crack (*C*) perpendicular to the fibre direction. In some instances of tests of this type, tensile failure near point *D* takes place.

The incorporation of carbon fibres into magnesia, glasses and aluminium is being investigated⁴⁵ and there is thus a possibility in the future of toughened ceramics and metals. One difficulty in the reinforcement of metals with carbon fibre is the tendency for a chemical reaction to take place. This difficulty can be overcome by coating the carbon fibres with a barrier layer of an unreactive third material (for example, tungsten⁴⁶).

Development Potential

Whiskers of graphite, prepared for example by a high pressure carbon arc technique⁴⁷, have extremely high tensile properties; tensile strengths of 3×10^6 pound inch⁻² and a Young's modulus of 100 to 130×10^6 pound inch⁻² have been achieved⁴⁷, but it has been estimated that the theoretical strength and modulus of the graphite crystal are 14.5 to

20×10^6 and 145×10^6 pound inch⁻², respectively⁴⁸. There is therefore ample room for development in the tensile properties of the acrylic based carbon fibre, particularly in the strength. Whiskers are, however, almost continuous crystals of graphite, whereas carbon fibres are polycrystalline and will therefore suffer from inherent limitations in strength and modulus as a result of the structural imperfections associated with the crystallite boundaries.

Carbon fibres made from acrylic precursor fibre are by no means the only form of carbon fibre available. Carbon fibres made from cellulose have been available for many years and, though of relatively modest tensile properties, they have attributes that make them suitable for a range of applications (for example, thermal insulation). Union Carbide have improved the orientation, and thus the mechanical properties, of cellulose carbon fibres by stretching them during graphitization at more than 2,000°C; these stress-graphitized fibres

(the Thornel fibres) have mechanical properties not too far below those of acrylic based carbon fibres.

Why have only acrylic and cellulose fibres been used on any large scale as precursors for carbon fibres? Although a complete answer to this question is not possible, the basis of the answer is probably reasonably well understood. The organic fibres that can be carbonized fit into two groups: those that give a carbon fibre that is graphitizable and those that give a carbon fibre that is not in reality these are probably two extremes, with all carbon fibres lying somewhere in between. The graphitizable carbon fibres probably have superior tensile properties (after heating to graphitizing temperatures) than the equivalently heated non-graphitizing carbon fibres⁴⁹. Carbon fibres from acrylic fibres are graphitizable and this is probably one reason for their superior tensile properties. But why are they the best of the currently available graphitizable carbon fibres? The answer probably lies, partly at least, in the high orientation of the acrylic based carbon fibres, which in turn originates in the particular nature of the chemical reactions taking place in the acrylic degradation process (principally in the oxidation stage under tension). These reactions stabilize and preserve the orientation during the later stages of the heating process.

Carbon fibres are being produced from precursors other than organic fibres, however. The Kureha Company in Japan, for example, makes carbon fibre from pitch. The Kureha fibres available are, however, of low orientation and have similar mechanical properties to glass fibres (that is, they are of comparatively low modulus). A research group at the University of British Columbia are also producing carbon fibres from low cost pitch materials: excellent mechanical properties (similar to those of acrylic based carbon fibres) have been reported for pitch based fibres that have been stretched during graphitization at 2,000°C to 2,800°C (ref. 50). The British Coal Utilization Research Association's Industrial Laboratories are making strong carbon fibres from coal products and fibres stronger than E-type glass fibre have successfully been prepared⁵¹.

Composite Applications

The most likely immediate applications for composites reinforced with carbon fibres are those in which their combination of strength, stiffness, and lightness is at a premium and in which the cost of the material is less important³⁴⁻³⁶. The aircraft and space industries meet both these conditions and will, no doubt, provide numerous applications in the future for carbon fibre reinforced plastic, despite the much discussed difficulties with the fan blades of the Rolls-Royce RB211 engine. It seems likely that several other aircraft components (helicopter blades, aircraft structure parts, aircraft containers and so on) will also eventually be made from carbon fibre reinforced plastic. The advantages of carbon fibre reinforced plastics over metals in space vehicles (for example, rocket motors and satellites) are particularly obvious because of the large amount of fuel needed to put a small weight into space.

The cost of carbon fibres, and difficulties in the use of composite materials for some purposes, have restricted the number of "earthbound" uses of high performance carbon fibre. At the Atomic Energy Research Establishment, Harwell, carbon fibre composites have been used in chopper disks in neutron experiments³ and Dowty Rotol Ltd make fan blades from the material⁵²; Bonas Brothers use carbon fibre reinforced plastic to make a special component for a high speed loom⁵³. The high strength and stiffness of RAE-type carbon fibre has also been utilized with benefit in the panels of the Ford GT40 sports car.

This present rather limited use of carbon fibre reinforced material is, however, a phase that will soon pass, and there is no doubt that in the not too distant future the use of the material will expand rapidly. Some possible applications include the manufacture of boats and submarines for which the fact that carbon fibre composites are little affected by water is a particular advantage and pressure vessels of all types. The relative chemical inertness of carbon makes the use of carbon fibre composites of considerable potential value in chemical plant environments that are severe, both chemically and physically⁵⁴. Any engineering device involving rotating members will obviously be improved if manufactured from carbon fibre reinforced plastic, because the centrifugal forces in the rotating part depend on the weight of the part. There are also improvements to be gained in the manufacture of wires and cables for high stress-bearing situations and engine components for high performance internal combustion engines. Composite material containing chopped fibre has also been shown to have excellent friction and wear characteristics⁴²⁻⁴⁴. The high strength and modulus of carbon fibre reinforced plastic allows a good balance to be achieved on sports equipment—adequate strength can be allied to a proper distribution of weight.

Research at Shirley Institute

Certain aspects of the development work on carbon fibres and composites have clearly required knowledge and expertise of a textile, rather than engineering, character. For this reason both the former Ministry of Technology and the former Rolls-Royce Ltd sponsored research work on carbon fibres at the Shirley Institute. This many sided research is confidential in nature and it is only possible to mention briefly two pieces of work. The first, performed under contract to the Ministry of Technology, is the development of a novel method for spinning yarns from the comparatively short lengths of carbon fibre produced by the batch process. Ordinary textile machinery and processing could not be used because the carbon fibres are too smooth and slippery. Work was also carried out under contract to the former Rolls-Royce Ltd on the development of a new weaving method for assembling filaments into a wide, flat, continuous sheet.

The Institute now possesses widely based expertise and facilities for research on carbon fibres and composite materials, and is continuing a high level of effort in this field, both in

its general research programme and in the rapidly expanding sponsored sector of its activities.

I am grateful to the Composite Section of Shirley Institute for the photomicrograph of the tested specimen of composite.

- ¹ Watt, W., Phillips, L. N., and Johnson, W., *The Engineer* (May 27, 1966).
- ² Johnson, W., Phillips, L. N., and Watt, W., *BP*, 1, 110, 791 (1968).
- ³ *Carbon Fibres* (edit. by Smith, E. A.) (Morgan-Grampian, London, 1970).
- ⁴ *Carbon Fibres* (Report from the House of Commons Select Committee on Science and Technology, HMSO, 1969).
- ⁵ Gunston, W. T., *Sci. J.*, 39 (February 1969).
- ⁶ *New Sci.*, 564 (June 13, 1968).
- ⁷ *Chemical and Engineering News*, 35 (August 26, 1968).
- ⁸ Shindo, A., *Rep. Govt. Ind. Res. Inst. Osaka*, No. 317 (1961).
- ⁹ Burlant, W. J., and Parsons, J., *J. Polymer Sci.*, 22, 249 (1956).
- ¹⁰ Miyamichi, K., et al., *J. Soc. Fibre Sci. Tech., Japan*, 22, 538 (1966).
- ¹¹ Turner, W. N., and Johnson, F. C., *J. Appl. Polymer Sci.*, 13, 2073 (1969).
- ¹² Watt, W., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
- ¹³ Watt, W., *Nature*, 222, 265 (1969).
- ¹⁴ Standage, A. E., and Prescott, R., *Nature*, 211, 169 (1966).
- ¹⁵ *Carbon Fibres for Reinforced Plastics* (Royal Aircraft Establishment, 1967).
- ¹⁶ Moreton, R., Watt, W., and Johnson, W., *Nature*, 213, 690 (1967).
- ¹⁷ *Fibre Data Summaries* (edit. by Ford, J. E.) (Shirley Institute Pamphlet No. 91, 1966).
- ¹⁸ Watt, W., *Interlab. Symp. Carbon Fibres*, 2 (1969).
- ¹⁹ Johnson, J. W., *Amer. Chem. Soc. Polymer Prepr.*, 9, 1316 (1968).
- ²⁰ Thorne, D. J., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
- ²¹ Brydges, W. T., Badami, D. V., Joiner, J. C., and Jones, G. A., *Amer. Chem. Soc. Polymer Prepr.*, 9, 1310 (1968).
- ²² Johnson, W., and Watt, W., *Nature*, 215, 384 (1967).
- ²³ Moreton, R., *Royal Aircraft Establishment Tech. Rep. 69114* (1969).
- ²⁴ Moreton, R., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
- ²⁵ Watt, W., and Johnson, W., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
- ²⁶ Watt, W., and Johnson, W., *Amer. Chem. Soc. Polymer Prepr.*, 9, 1245 (1968).
- ²⁷ Johnson, J. W., Marjoram, J. R., and Rose, P. G., *Nature*, 221, 357 (1969).
- ²⁸ Logsdail, D. H., *Amer. Chem. Soc. Polymer Prepr.*, 9, 1324 (1968).
- ²⁹ Johnson, W., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
- ³⁰ Johnson, D. J., and Tyson, C. N., *Brit. J. Appl. Phys. Phys. Ser. 2*, 2, 787 (1969).
- ³¹ Sanderson, D. H., and Windsor, C. G., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
- ³² Spencer, D. H. T., Hooker, M. A., Thomas, A. C., and Napier, B. A., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
- ³³ Johnson, D. J., and Tyson, C. N., *Proc. Roy. Microsc. Soc.*, 4, 19 (1969).
- ³⁴ Phillips, L. N., *Royal Aircraft Establishment Tech. Rep. 67088* (1967).
- ³⁵ Phillips, L. N., *Fibre Sci. Tech.*, 1, 3 (1968).
- ³⁶ Phillips, L. N., *Composites*, 1, 101 (1969).
- ³⁷ Pearce, D., *Interlab. Symp. Carbon Fibres*, 8 (1969).
- ³⁸ Dauksys, R. J., and Ray, J. D., *J. Comp. Materials*, 3, 684 (1969).
- ³⁹ Morley, J. G., *Phys. Bull.*, 20, 479 (1969).
- ⁴⁰ Milewski, J. V., Shaver, R. G., and Withers, J. C., *Materials Engineering* (May 1968).
- ⁴¹ Prosen, S. P., and Simon, R., *Reinforced Plastics and Composites World* (September/October 1967).
- ⁴² Lancaster, J. K., *Proc. Inst. Mech. Eng.*, 182, Pt. 1, 33 (1967/68).
- ⁴³ Giltrow, J. P., and Lancaster, J. K., *Nature*, 214, 1106 (1967).
- ⁴⁴ Giltrow, J. P., and Lancaster, J. K., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).
- ⁴⁵ *The Engineer*, 499 (March 29, 1968).
- ⁴⁶ *Fulmer Research Institute Newsletter* (June 1969).
- ⁴⁷ Bacon, R., *J. Appl. Phys.*, 31, 283 (1960).
- ⁴⁸ McCreight, L. R., Rauch, sen., H. W., and Sutton, W. H., *Ceramic and Graphite Fibres and Whiskers*, 56 (Academic Press, 1965).
- ⁴⁹ Badami, D. V., *New Sci.*, 45, 251 (1970).
- ⁵⁰ Hawthorne, H. M., Baker, C., Bentall, R. H., and Linger, K. R., *Nature*, 227, 946 (1970).
- ⁵¹ *The Engineer*, 30 (July 2, 1970).
- ⁵² Peters, D. M., *Design Engineering*, 104 (February 1969).
- ⁵³ *The Engineer*, 38 (November 13, 1969).
- ⁵⁴ Judd, N. C. W., *Soc. Chem. Ind. Conf.: Industrial Carbons and Graphite* (1970).

New Hominid Skull from Bed I, Olduvai Gorge, Tanzania

M. D. LEAKEY, R. J. CLARKE & L. S. B. LEAKEY

Centre for Prehistory and Palaeontology, PO Box 30239, Nairobi

Restoration of a hominid skull recovered from Bed I at Olduvai Gorge has revealed it to be a member of the genus *Homo*. Although the new cranium resembles *H. habilis* from Bed II, it differs in some respects from the type of *H. habilis* from Bed I. This may be a case of sexual dimorphism.

THE cranium described by L. S. B. L. later in this article (Olduvai Hominid 24) was found by P. Nzube in October 1968 (ref. 1) at a site in Olduvai Gorge that lies in the eastern part of the gullies known as DK, approximately 300 m east of the living site in lower Bed I that was excavated during 1962 and 1963.

The Site

The cranium was embedded in a mass of calcareous matrix measuring approximately $20 \times 12 \times 8$ cm. All the bones were covered by matrix with the exception of a small portion of the right supra-orbital region and the posterior part of the palate. Indeed, so few identifiable parts were visible that it is remarkable it was recognized as hominid. The deposit on which it was found consists of a tuffaceous clay that can be traced over a wide area in Bed I, extending from the eastern part of the Gorge as far west as the FLK sites. This horizon overlies the basalt and is in turn overlain by the marker tuff IB for which an apparently reliable average date of 1.8 m.y. has been obtained by K/Ar dating². At the hominid site (DK East), the tuffaceous clay is 3 m thick but the thickness varies considerably at different localities, depending on the surface configuration of the underlying lava. The clay at several other sites has yielded artefacts and fossil bones, including the molar tooth of Olduvai H. 4, found at MK during 1959, that has been attributed to *Homo habilis*³ as well as the only remains of a chalicothere known from Olduvai.

After the discovery of the cranium the surface deposit was sieved extensively and an area of approximately 400 m² was eventually worked over. Three complete and some broken teeth were recovered as well as substantial portions of the maxillae, part of the occipital, several fragments of the parietals and the left supra-orbital region of the frontal bone. Some fragments were found low down the erosion slope and at a depth of just under 1 m from the present ground level: others lay on the surface, close to the block of matrix containing the cranium. It is evident that the specimen had been exposed for many years and that, as fragments broke off, some rolled down the slope and were buried under hill wash, while others

became detached more recently and remained on the surface near the parent block. No trace of the canine and incisor teeth was found and it is likely that they broke off first and were transported downhill by erosion, possibly as far as the present river course.

The matrix in which the cranium was embedded is indistinguishable from the limestone concretions that frequently occur within the tuffaceous clay that overlies the basalt at DK and elsewhere (the mandibular fragment containing the molar of Olduvai H. 4 was similarly embedded). These concretions often form round fossil bones that can be seen as central cores when the blocks are broken open.

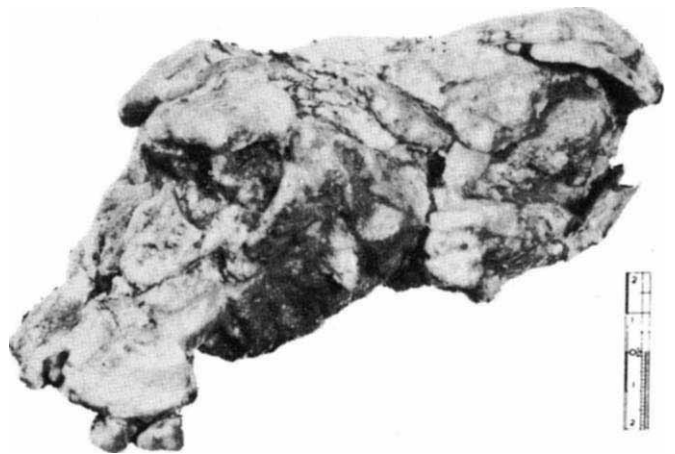


Fig. 1 Cast of Olduvai H. 24 after partial cleaning and before reconstruction.

Crocodile remains predominate among the faunal material from this site and more than 2,000 teeth were found. Tortoise plates, shells of Urocyclid slugs, fish vertebrae and scales, bird bones and pieces of ostrich eggshell were also relatively common. Mammalian bones and teeth included those of primates, rodents, carnivores, equids, suids, giraffids and bovids. There were also more than 37,000 unidentifiable bone fragments. The state of preservation of these remains is very similar to that of the cranium and many were also found embedded in limestone concretions.

Associated Stone Industry

Apart from a few quartzite flakes and chips, no artefacts were found, but the living floor previously excavated at DK—which included the circle of lava blocks considered to represent an artificial structure—has yielded a large series of artefacts

that represent the contemporary stone industry⁴. This site consists of a living floor within the same tuffaceous clay that occurs at DK East and is similarly overlain by Tuff IB. In some places the accumulation of occupation debris rests on the surface of the lava, so that the living floor is at a slightly lower level than the layer of limestone concretions from which the cranium is believed to have eroded. Artefacts recovered from the living floor and from the clay above amount to 1,198 specimens, consisting of 154 tools, 187 utilized pieces and 857 debitage. The industry is typical of the Oldowan as it occurs throughout Bed I. It includes choppers of various forms, generally made on water-worn lava cobbles, polyhedrons, discoids, sub-spheroids, heavy and light duty scrapers and burins. Although the tools are of the usual Oldowan types, a proportion of the choppers, polyhedrons and discoids are smaller than those from higher levels of Bed I.

The associated faunal material includes all the species noted at DK East with the addition of *Elephas* and *Deinotherium*. Crocodile remains are similarly the most numerous.

Restoration of Olduvai H. 24

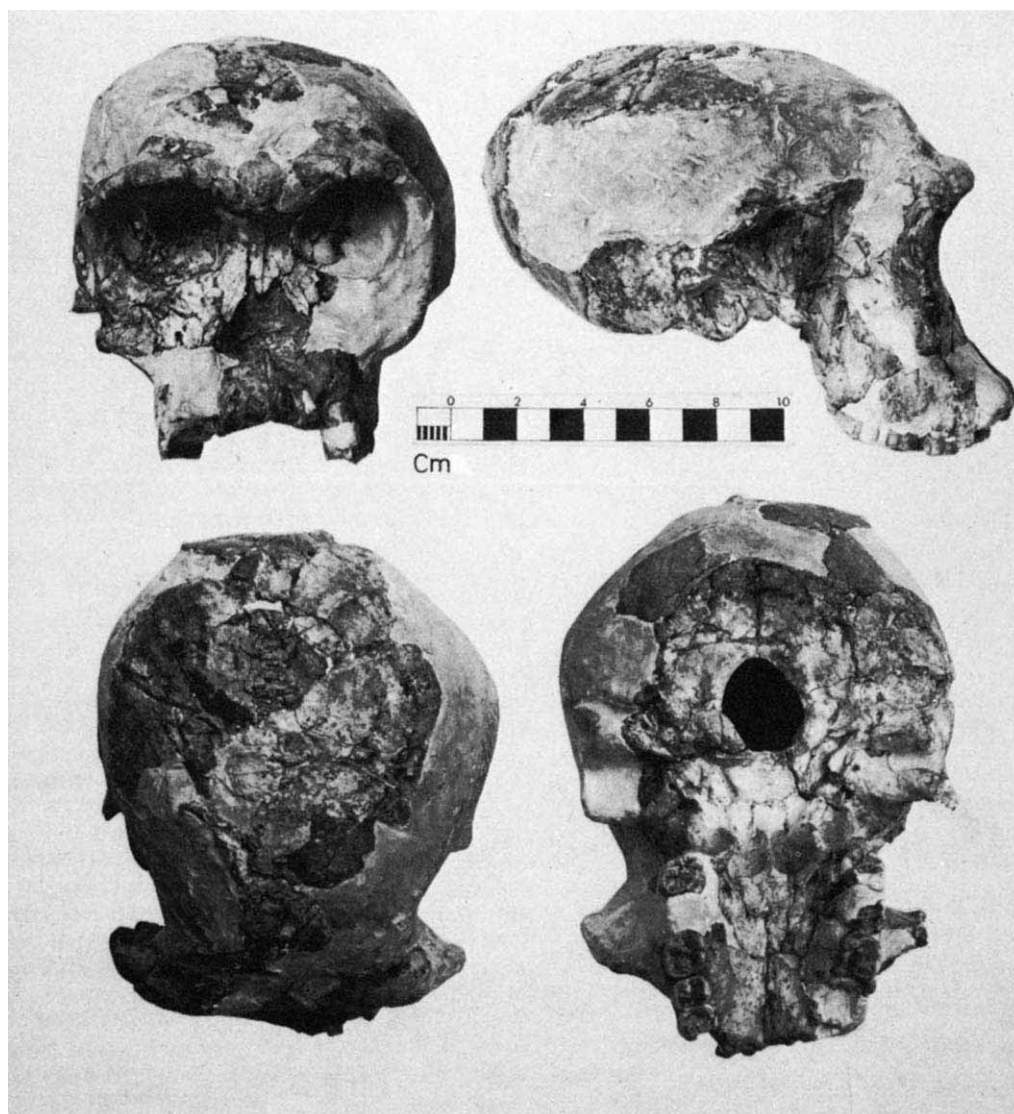
The tools used by R. J. C. in the removal of matrix from Olduvai H. 24 were a chisel-ended dental pick and a small hammer, a diamond drill and an S. S. White Industrial Air-Abrasive machine. Acetic acid could not be used because although the exposed pieces of bone had become hardened by weathering, those not exposed were softer than the matrix.

Parts of the matrix contained impressions of bone fragments that had fallen out during weathering. Some of these were found during excavation and it was possible to fit them back into position and thus build up the cranium into the form it had been before they became detached. It was apparent that the specimen was reasonably complete but had been badly crushed and distorted, the chief pressure having been downward and backward from the brow region, with the result that the nasal bones, although complete and perfectly preserved, were lodged down behind the infra-orbital region. The glabella, still attached to the nasals, was crushed, and the brow ridges were squeezed close together on top of the glabella.

The vault fragments were first separated and then completely cleaned of matrix under a binocular microscope. The facial fragments were more difficult to separate because they were interlocked and rammed against each other, but once they were free of matrix there were perfect joins between the nasals and the rest of the face and between the right brow ridge and the right orbital margin, just above the fronto-zygomatic suture.

The base of the cranium had been depressed into the brain cavity and was rammed against the right petrous, and the basi-occipital had been pushed under the vomer.

After thorough cleaning, consideration of sutures, bone thickness and curvature it was found that all the vault fragments but one joined together and that there was contact from the foramen magnum almost to the glabella. The sides of the vault, however, were not present. The posterior part was



considerably crushed and in order for the joins in this region to lock properly it was necessary to straighten out the buckled lambdoid region by breaking through the bone along one crushed bend and by partially cutting and breaking the bone just above the suture.

The crowns of the left P^4 , M^1 and right M^1 that were found during sieving fitted back directly on to the palate, but the left P^3 and the fragment of right M^2 had to be placed in position with plaster supports. A small but perfect contact between the mesiolingual fragment of the right M^3 and the major portion of this tooth could be seen under the microscope. The two pieces were fitted together and were placed in position on the palate by means of an impression that had been left in the matrix.

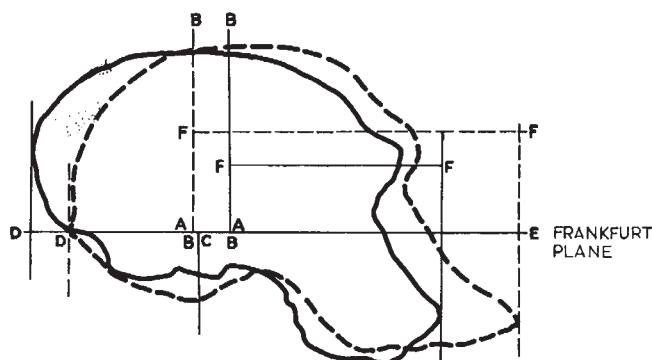


Fig. 3 Profile of Olduvai Hominid 24 (solid line) superimposed on that of *Australopithecus africanus* ST. 5 (broken line).

The cranium is still warped slightly and it is doubtful whether it can be completely straightened out, particularly as the sides of the vault are missing. The right petrous and tympanic plate are still in their crushed position and it would be a major operation to attempt to restore these bones to their proper relationship. The base of the cranium is also still depressed into the brain cavity and its thinness in places and the fact that it is covered with a network of fine cracks inhibit the possibility of restoration. The crushing of the whole cranium has also to be taken into account when considering the cranial capacity, which must inevitably have been greater than the absolute capacity as measured now.

Description of Olduvai H. 24

This specimen now consists of the following parts: almost the entire supra-orbital region of the frontal, the greater part of the vault, a considerable part of the occipital bone, nearly all the sphenoid, nearly all the right temporal bone and a considerable part of the face and palate, including the nasal bones. (Measurements are given in Tables 1 and 2.)

(1) The frontal bone. The supra-orbital region on the right side is almost intact as well as most of the glabella region. A large part of the left supra-orbital region is also preserved but it does not extend quite as far as the external orbital angle. There is also a small piece missing between the left supra-orbital fragment and the glabella region. On the right side the external orbital angle is intact and in normal articulation with the orbital process of the zygomatic. The supra-orbital region as a whole, while forward projecting, is not very massive. The glabella contact with the nasal bones at nasion is preserved and nasion lies well behind the overhanging glabella. Behind the supra-orbital region there is a depressed valley that is more pronounced above the glabella than on either side. In such parts as are preserved the frontal bone rises from this valley in a low curve towards bregma. There is sufficient of the frontal bone to be reasonably certain of the glabella-bregma length, which is approximately 90 mm.

On the right side of the cranium the region immediately behind the external orbital angle and the supra-orbital torus is well preserved. Part of this area is also present on the left side so that the minimum frontal width can be closely estimated. The figure is 75 mm.

(2) The parietals. Although neither of the two parietal bones is intact, the whole of the sagittal suture is preserved so that the bregma-lambda cord and arc can be measured with reasonable accuracy: these are 76.5 mm and 81.0 mm respectively. On either side of the sagittal suture the parietal bones extend laterally with only a slight downward curve for about 50 mm. Although the lateral parts of both parietals are missing, these bones must then have turned abruptly downwards if they were to meet the squamous parts of the temporal bones. This can clearly be seen on the more complete right side of the cranium. The sagittal suture is very largely fused but can still be traced. This condition is somewhat surprising in an individual whose third molars were barely erupted and not yet in occlusion. It suggests that the eruption of the third molars in early hominids based on analogy with modern man may not be a reliable indication of age.

(3) The occipital bone. A large part of this bone is well preserved, especially on the right side. There is enough of the nuchal area intact as well as continuous contact from lambda to opisthion to make a reasonable reconstruction of the upper half of the occipital and it is possible to estimate, with a fair degree of accuracy, the lambda-opisthion arc and cord: these measure 87.5 mm and 71.0 mm respectively. The arc/cord index (Martin's index 125)⁵ is 81.14. By doubling the measurement from the right asterion to the midline of the occipital it is also possible to estimate the bi-asterionic width, which is approximately 99 mm. The occipital index (Martin's index 129) is approximately 72.0.

Viewed in profile, when the skull is orientated in the Frankfurt plane, the occipital region is seen to project far beyond a vertical line through the external auditory meatus, a feature seen in *Homo* but not in *Australopithecus*. The occipital condyles lie between the external auditory meati and the foramen magnum is elongate. The basi-occipital projection is short.

Table 1 Measurements of Olduvai H. 24 Cranium (in mm)

Glabella toinion cord	147.0
Glabella to bregma cord	88.5
Bregma toinion cord	78.5
Bimastoid crest breadth	122.0
External orbital angle width	107.0
Minimum frontal width	75.0
Foramen magnum length	28.5
Foramen magnum width	25.0
Length nasal bones	19.5
Nasion to prosthion	60.0
Nasion to orale	61.0
Nasion to left side of pyriform aperture	38.5
Nasion to naso-spinale	36.0
Naso-spinale to prosthion	25.0
Maximum breadth of pyriform aperture	25.0
Palatal width at alveolae of M^1	37.0

Table 2 Measurements of Teeth of Olduvai H. 24 (in mm)

Left side	Bucco-lingual breadth	Mesio-distal length
P^3	(12.0)	9.0
P^4	(12.5)	9.1
M^1	(13.0)	12.6
M^3	(14.5)	12.0

(4) The sphenoid bone. Nearly all the sphenoid is preserved including the pterygoid plates but not the pterygoid hamuli. In general appearance this bone is very similar to that seen in *Homo*, but this area requires detailed study in order to elucidate its significance taxonomically.

(5) The temporal bones. In the right temporal only the superior portion of the squamosal and part of the zygomatic process are missing. The mandibular fossa is perfectly preserved and is deep and narrow. The external auditory meatus is large and rather parallel-sided. The posterior part of the tympanic plate rises steeply to separate the meatus from the mastoid region. The tip of the mastoid process is broken off and the original size can only be estimated approximately from the preserved part. The zygomatic process is slenderly built and lacks the wide lateral shelf and outward flaring generally seen in *Australopithecus*.

The greater part of the left temporal bone is missing except for a small fragment in the medial region of the mandibular fossa.

(6) The face and palate. (a) The nasal bones. Both nasal bones are preserved with only the tips of the lateral processes on either side slightly damaged. The contact of the two bones in the midline is marked by a distinct keel which continues almost to the end of the nasal area. The margins of the pyriform aperture are not quite intact, but at its widest point it measures approximately 25 mm. The nasal length from nasion to the left lateral lip of the nasal aperture is 42.5 mm and nasion to nasospinale 36.0 mm. The nasal index (Martin's index 148) is 69.4, that is hyperchamaerhine. There is a well developed anterior nasal spine at the centre of the pyriform aperture and from this point a very distinct keel runs downwards in the mid-line on the naso-alveolar clivus. (b) The zygomatic bones. The right zygomatic bone is reasonably well preserved; only the zygomatic process and a chip from the frontal process are missing. The root of the zygomatic arch lies approximately above a point between the first and second molars. The left zygomatic bone is missing. (c) The maxillae. The right maxilla is almost complete; parts of the alveolar process are missing, particularly in the region of M², and at the canine jugum the bone is also missing. The left maxillary bone is less complete, the zygomatic process, part of the nasal surface, and much of the posterior part of the alveolar process are missing. At the incisor sockets the alveolar margin seems to be preserved lingually but is broken labially. At present the margin runs straight across between the two canine alveoli and is not curved; but it is difficult to assess the original form on account of the damage. The whole of the median palatal suture is preserved. The right side of the bony palate has been displaced posteriorly by approximately 2 mm. The palate is seen to be reasonably deep (depth of alveolar processes at the first molars is approximately 15.0 mm). (d) The palatine bones. The palatine bones are intact and the areas of their contact with the maxillary tuberosities and with the sphenoid are well preserved.

(7) The teeth (see Table 2) present in the cranium as now reconstructed are as follows: (a) The crown of the left M³. This is slightly broken on the distal aspect. The occlusal surface is undamaged and is very wrinkled with no sign of wear. (b) The left M¹ is intact and is firmly rooted in the maxilla. The enamel of the crown shows a degree of flat wear and the dentine is only exposed in a very small area on the protocone. (c) The left P⁴ is complete. Its bucco-lingual width is less than that of M¹ and it is rather rectangular in form. (d) The left P³ is partially preserved, the mesio-lingual area is broken away. (e) the right M³ is partially preserved, the paracone and part of the protocone are broken away. The tooth compares closely with the intact left M³. (f) The right M² is incomplete; it is broken diagonally so that the lingual and distal area is missing. (g) The right M¹ is nearly complete, only the protocone is missing. Its wear and structure are like those of the left first molar.

In addition to these teeth several fragments of molars and

premolars were also recovered during sieving. It has not yet been possible to fit these fragments on to the cranium.

(8) Cranial capacity. It is not possible at this stage to give a close estimate of cranial capacity but preliminary determinations give a figure in the order of 560 cm³.

(9) Endocranial region. Considerable parts of the endocranial morphology are preserved.

Comparisons with Other Hominids

There is still some evidence of distortion in the reconstruction. This has resulted in the vault of the skull being lower than it was originally and the backward projection of the occipital is now exaggerated.

The frontal bone does not exhibit the marked post-orbital constriction that is seen in the australopithecines from South and East Africa, nor does the supra-orbital element of the frontal bone curve backwards to the same extent. Seen in both lateral and vertical views the supra-orbital torus and glabellar region form a more or less straight line whereas in all known species of *Australopithecus* the glabella tends to project further forward with the lateral edges of the supra-orbital torus swinging backward. The parietal bones extend outwards from the sagittal suture with only a slight downward curve at first, and then bend steeply downwards to meet the squamosal parts of the temporal bones. The parietal region of the brain is thus more expanded than in *Australopithecus*, where the parietals usually begin to curve downwards close to the mid-line. Even allowing for the backward distortion of the occipital region this area of the skull extends further behind a vertical line through the external auditory meatus, when the cranium is in the Frankfurt plane, than in the australopithecines. The mandibular fossae are deep and very similar morphologically to those of *Homo sapiens* and wholly unlike those seen in *Australopithecus*. The nasal bones are long and slender with a marked keel in the mid-line.

The discovery of this skull, which is clearly a member of the genus *Homo* as defined in 1964⁶, although it has certain primitive morphological features, raises an important issue. Although it is clearly not an australopithecine it nevertheless differs in certain important respects from the incomplete type of *Homo habilis* (Olduvai H. 7). Yet it resembles the paratype from Bed II (Olduvai H. 13) in both cranial and dental characters. At the same time, the dentition of the type of *Homo habilis* shows considerable resemblances to that of Olduvai H. 16, from lower Bed II, Maiko Gully, although differing in some respects. The problem therefore is whether or not there are two forms of *Homo* in Beds I and II or whether the differences between the two groups can be accounted for on the basis of individual variation and sexual dimorphism.

Although only part of the upper dentition is preserved in the cranium from DK, there are other specimens from Beds I and II which have a comparably small or even smaller tooth size and are morphologically similar. These clearly belong within the same group. They include a complete upper molar (Olduvai H. 6) from the site of *Australopithecus boisei* and an isolated molar (Olduvai H. 21), also probably from Bed I.

In Olduvai H. 24 from DK and in H. 13 from MNK certain important diagnostic characters of the skull are preserved, including the area of the temporal bones around the external auditory meatus and most of the occipital bones. These features, together with the morphology of the teeth, clearly indicate that both specimens belong within the genus *Homo*.

Although the occipital bone of the type specimen of *Homo habilis* (Olduvai H. 7) is not preserved it is evident from the structure of the parietal bones, and especially the form of the lambdoid suture, that the occipital bone must have been morphologically similar to that of *Homo erectus*, that is to say, the lambdoid suture is in the form of a low, wide arch and not V-shaped. This form is also seen in the skull of Olduvai H. 16 from lower Bed II. In contrast, the form of this suture in both Olduvai H. 24 from DK and Olduvai

H. 13 from MNK and, indeed, the whole form of the occipital bone, is very similar to that seen in *Homo sapiens* today: the line of the suture descends sharply from lambda in the form of an inverted V. There are thus two types of occipital morphology, one represented by Olduvai H. 24 and Olduvai H. 13 and the second by the type of *Homo habilis* and Olduvai H. 16.

The teeth of Olduvai H. 7 and H. 16 are megadont and within the size range of *Australopithecus africanus*; yet, in other respects, they display a number of morphological differences, especially in the premolars.

Distinct differences from the australopithecines are also evident in the cranial morphology, particularly in the parietals. Although the frontal bone of the type of *Homo habilis* is missing, a large part of this bone is preserved in Olduvai H. 16. Both the minimum frontal width and the width between the temporal crests is greater than in any known australopithecine.

Until the discovery of Olduvai H. 24 it was considered possible that the difference in morphology and in size between the cranial parts and dentition of the type of *Homo habilis* from Bed I and those of the paratype from Bed II (Olduvai H. 13) might have resulted from the elapse of a prolonged time interval. Because Olduvai H. 24 is chronologically near to the type of *Homo habilis* and yet more closely resembles the paratype from Bed II this explanation is no longer tenable. There remains the question of sexual dimorphism and it is

possible that Olduvai H. 13 and H. 24 represent females of *Homo habilis* with the type and H. 16 representing the males. The difference in the morphology of the occipital region in the two groups, however, cannot be disregarded and suggests the possibility of taxonomic variation.

Beyond doubt, the new specimen represents the genus *Homo* as defined by Leakey, Tobias and Napier and differs fundamentally from the australopithecines. At the same time it is not entirely certain that it necessarily represents a female of *Homo habilis*.

We thank Dr A. Walker of the University of Nairobi for checking the text and measurements and for most valuable comments.

Received April 2, 1971.

¹ Leakey, M. D., *Nature*, **223**, 754 (1969).

² Curtis, G. H., and Hay, R. L., in *Calibration of Hominoid Evolution* (edit. by Bishop, W. W., and Miller, J.) (Scottish Academic Press for Wenner Gren Foundation for Anthropological Research, in the press).

³ Leakey, L. S. B., and Leakey, M. D., *Nature*, **202**, 3 (1964).

⁴ Leakey, M. D., in *Background to Evolution in Africa* (edit. by Bishop, W. W., and Clark, J. D.) (University of Chicago Press, Chicago and London, 1967).

⁵ Martin, R., *Lehrbuch der Anthropologie* (Fischer, Stuttgart, 1959).

⁶ Leakey, L. S. B., Tobias, P. V., and Napier, J. R., *Nature*, **202**, 5 (1964).

Non-random X Chromosome Expression in Female Mules and Hinnies

J. L. HAMERTON, B. J. RICHARDSON & PHYLLIS A. GEE

Department of Medical Genetics, Children's Hospital, Winnipeg, and Departments of Paediatrics and Anatomy, University of Manitoba

W. R. ALLEN & R. V. SHORT

ARC Unit of Reproductive Physiology and Biochemistry, Animal Research Station, Huntingdon Road, Cambridge, and Department of Veterinary Clinical Studies, School of Veterinary Medicine, Madingley Road, Cambridge

The behaviour of X-linked glucose-6-phosphate dehydrogenase locus in a mule and two hinnies provides support for Lyon's single active X hypothesis.

THERE have been several studies of X chromosome DNA replication and the behaviour of X-linked genes in the female mule¹⁻⁶ but the results are not in agreement on the frequency with which the two parental X chromosomes (X^D and X^H) show late DNA replication. Here we present additional data on the expression of the X-linked glucose-6-phosphate dehydrogenase (*Gd*, E.C.1.1.1.49) locus in a female mule and two female hinnies. (The hinny is the reciprocal cross *Equus caballus* ♂ × *E. asinus* ♀.) The mule and one hinny were less than one year old and the third animal was a 35 day old hinny foetus obtained by hysterotomy. All were bred at the Veterinary School and ARC Unit of Reproductive Physiology and Biochemistry, Cambridge, England.

Fibroblast cultures were established from the three animals by standard methods. The medium used was McCoy's 5a (modified) supplemented with 15% foetal calf serum (McFC15). Cloning of fibroblasts was carried out as follows⁷. Healthy exponentially growing fibroblast cultures were trypsinized, and the cell suspension was diluted as necessary to a final dilution of about thirty cells per ml. One drop of this suspension (0.03 ml.) was placed in each well of an appropriate number of Falcon Microtest II culture plates. (Each plate contains ninety-six wells of 0.5 ml. capacity. For each experiment five or six plates were seeded.) Then 0.2 ml. of McFC15 was added to each well and the plates were covered and incubated in a 100% humidity incubator in an atmosphere of 5% CO₂ in air. They were examined after 3 and 5 days and wells containing one colony were marked. The plating efficiency varied from 20-40%, which was similar to that obtained in conventional plating experiments using the same cell suspensions.

After 10-14 days, wells containing single colonies were trypsinized and the cell suspension transferred to 60 mm Falcon Petri dishes. When these dishes were confluent, the cells were either transferred to culture bottles to allow more

extensive growth or, alternatively, the G6PD types were determined on the cells in the Petri dishes without further passage. Cells from each original culture and from a number of clones were stored at -70 or -196°C in a cryoprotective medium containing 15% glycerol. Lysates were prepared from both original cultures and clones by freezing and thawing in 0.015 M Tris-borate buffer (pH 7.8) containing 0.005 M EDTA, 0.02 g% 'Triton X-100', 2 mg% NADP and 0.05% (v/v) 2-mercaptoethanol. Electrophoresis ('Cellogel', Chemtron, Milan) was carried out for 1.25 h at 4°C at a potential gradient of 45 V/cm and the gels were stained for glucose-6-phosphate dehydrogenase⁵.

were cloned are included in this table and show the expected *Gd* patterns. The interrelationship of the various hybrids which we have studied in detail is shown in Fig. 2. The hybrids, with one exception, develop a strong *Gd^H* band and either a faint or no *Gd^D* band. The exception (animal 192) has a stronger *Gd^D* band than *Gd^H* band. This inter-animal variation may explain the lack of agreement between different workers^{3,4}.

The *Gd* results obtained on 303 clones derived from three hybrids are given in Table 2. Two hundred and ninety-seven of these clones showed either the *Gd^H* or *Gd^D* band. Six clones were mixed (*Gd^H/Gd^D*), two of these were recloned, and 106 out of 107 of these sub-clones showed either *Gd^H* or *Gd^D*.

Table 1 Expression of Glucose-6-phosphate Dehydrogenase in Horses and Donkeys and their Hybrids

Animal No.	Type	Material	<i>Gd^D</i>	<i>Gd^H</i>	Source
Female hybrids					
100	Mule	E	±	+	Cambridge *
		F	—	+	
138	Hinny	E	±	+	Portugal
139	Hinny	E	—	+	Portugal
143	Mule	E	—	+	Portugal
187	Hinny	E	±	+	Portugal
188	Hinny	E	±	+	Portugal
189	Hinny	E	±	+	Portugal
190	Hinny	E	±	+	Portugal
		F	—	+	
191	Hinny	E	—	+	Portugal
192	Hinny	E	+	±	Portugal
195	Mule (foetus)	F	—	+	Cambridge †
196	Mule (foetus)	F	—	+	Cambridge †
200	Hinny (foetus)	F	—	+	Cambridge †
202	Mule	E	—	+	Cambridge
		F	±	+	Cambridge
203	Hinny	F	±	+	Cambridge
		E	±	+	
217	Mule	E	±	+	Cambridge
Male hybrids					
	Mule (n=1 foetus)	F	—	+	Cambridge
	Mules (n=4)	E	—	+	Portugal and Cambridge
	Hinnies (n=7)	E	+	—	Portugal and Cambridge
Parental species					
Males					
	Donkeys (n=7)	E	+	—	Cambridge
	Horses (n=2)	E	—	+	Cambridge
Females					
	Donkeys (n=12)	E	+	—	Cambridge
	Horses (n=8)	E	—	+	Cambridge

* This animal (No. 100) reported by Hamerton *et al.*, 1969.

† 195 45D gestation
196 60D gestation
200 35D gestation
201 ♂ mule 35D gestation
All obtained by hysterotomy

+ Strong; ±, faint; —, absent; E, erythrocytes; F, fibroblasts.

Gd is seen as a single band in both parental species, *Gd^H* moving slower than the *Gd^D* (refs. 2 and 8). The faint slower bands seen in both parental species and the hybrids after starch gel electrophoresis represent breakdown products. The earlier samples from animals 100–196 were typed on starch as described earlier². A number of other hybrid animals of both sexes as well as the parental species have been studied using both erythrocytes and fibroblasts without cloning. The *Gd* types are summarized in Table 1, and clearly confirm X-linkage of *Gd* in the Equidae. The parents of the three hybrids that

while one was again mixed (Table 3). In all animals, irrespective of the cross, the frequency of the two types is significantly different from 50% ($P > 0.0005$). This results from a deficiency of clones showing *Gd^D*.

Recloning the two mixed clones at a later passage showed that the proportion of *Gd^D* sub-clones (0.16) is significantly lower than expected, if it is assumed that these mixed clones originated from two cells, one *Gd^D* and one *Gd^H*. This suggests powerful selective forces acting *in vitro*.

A number of conclusions can be drawn from these results.

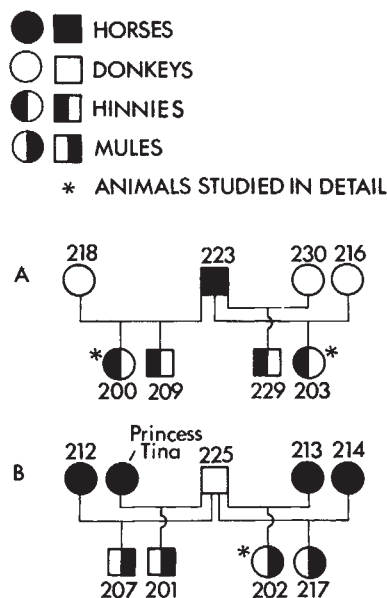


Fig. 1 Pedigree charts of the mules and hinnies studied. The numbers refer to numbers in Table 1. A, Hinnies; B, mules.

First, the mixed expression seen in the hybrids and some of the original cultures derived from them can be accounted for by the expression of one *X* chromosome only in any given cell and its descendants, as postulated by Lyon^{9,10}, and not from partial activity of both *X* chromosomes as postulated by Grüneberg¹¹ in his complementary *X* hypothesis. It could be argued, however, that the minor allele is expressed in less than 10% of the cells, in which case it would not be detected by the methods used here without sub-cloning. It seems unlikely that in a system where if the expression of an allele is equated to 100, all relative activities from 100 : 0 to 0 : 100 can reversibly occur for two alleles as suggested by Grüneberg¹¹, we would expect to find 81.7% of one type, 16.6% of the other and only 1.7% mixed, which are the results obtained here. Second, it can be concluded that active cell selection over a fairly short period of time occurs during *in vitro* fibroblast culture and that this selection would usually seem to be against those cells expressing *Gd^P* irrespective of whether the *X^P* is maternal or paternal in origin. This indicates that the maternal cytoplasm does not preferentially activate the maternal *X* chromosome or effect subsequent cell selection in these hybrids. Third, studies of *X*-linked loci using electrophoretic methods seem to demon-

strate activity of a given locus only when it is expressed in more than 10% of the cell population. Original cultures from animals 202 and 203 both showed a minor fast band (*Gd^P*) at an early passage; this was subsequently lost during culture. In both these animals the frequency of *Gd^P* cells as demonstrated by the typing of clones was between 0.1 and 0.3 in different experiments. The original cultures from the foetus (200) never showed a fast *Gd^P* band; in spite of this, 9.0% of clones showed expression of *Gd^P*. These results are interesting in the light of the results reported by Sharman and his co-workers¹²⁻¹⁴ on *X*-inactivation in marsupial hybrids in which they demonstrated preferential late replication of the paternal *X* chromosome and inactivation of the paternal *Gd* and phosphoglycerokinase (*PGK*) loci. Finally, the results obtained here agree with other data showing that the proportion of late replicating *X^P* ranged from 0.67-1.00 in six female mules and eight female hinnies⁶. If late replication of a given chromosome represents its genetic inactivation, then the autoradiographic results suggest that the proportion of cells expressing *Gd^P* should vary between 0.33 and zero which fits very closely to the results obtained here. Similar results have recently been reported for four mules by Cohen and Rattazzi⁵.

Table 3 Expression of *Gd* Locus in Sub-clones from Two Mixed (*Gd^PGd^H*) Clones from Animal 202

Clone	Expt.	No. <i>Gd^P</i>	No. <i>Gd^PGd^H</i>	Total sub-clones	Proportion <i>Gd^P</i>
C1	XIV	9	1	58	0.155
C8	XV	8	—	49	0.163
Total		17	1	107	0.159

These findings support the single active *X* hypothesis originally put forward by Lyon^{9,10}. They demonstrate clearly activity of only one *Gd* allele in any given cell, which has also been shown for man^{15,16}. Unfortunately, the other *X*-linked enzyme systems *PGK* and α -galactosidase which we have tested have failed as yet to show different mobilities in horse and donkey and so cannot be tested. Furthermore, these data demonstrate *in vitro* cell selection in favour of those cells carrying an active *X^H*. Examination of uncloned fibroblasts and erythrocytes from these animals also shows predominant expression of *Gd^H* (Table 1) suggesting that the proportion of cells with an active *X^H* *in vivo* are reflected in our *in vitro* results and that these are not therefore due to differential plating efficiencies of the two cell types. The one animal showing equal expression of the two types is unavailable for further study. Lyon^{9,10} has postulated *in vivo* cell selection to account for variation in expression of sex linked loci in the mouse and man and the demonstration of such strong selective forces *in vitro* due to the activity or inactivity of a single *X* chromosome lends support to the hypothesis that similar selective forces may be acting *in vivo*. Finally, the close agreement between the autoradiographic data^{2,4-6} and the genetic data presented here adds further support to the hypothesis that late replication is the cytological manifestation of genetic inactivity. A final proof of this would be provided by autoradiographic studies on the two clonal types derived here. These studies are at present in progress.

We thank Mrs G. Anderson, Mrs E. Cameron and Miss V. Niewczas-Late for technical assistance. This work was supported by a grant to J. L. H. from the Medical Research Council of Canada. Financial support from the Children's Hospital Research Foundation of Winnipeg is also gratefully acknowledged. W. R. A. is supported by the Thoroughbred Breeders Association. Collection of material from some of the animals in Portugal was supported by a grant to J. L. H. and R. V. S. from the Wellcome Trust. We thank Dr M. J. Freire and his veterinary colleagues for Portuguese samples.

Table 2 *Gd* Expression in Clones derived from Fibroblasts from Three Equine Hybrids

Animal No.	Expt.	No. <i>Gd^P</i>	No. <i>Gd^PGd^H</i>	Total	Proportion <i>Gd^P</i>
200 (Hinny ♀)	VIII	4	—	40	0.100
35 D Foetus	X	6	1	68	0.088
Total		10	1	108	0.093
202 (Mule ♀)	II	5	3	11	0.454
	IV	3	—	20	0.150
	VI	7	—	41	0.171
	XII	14	1	42	0.333
Total		29	4	114	0.254
203 (Hinny ♀)	VII	1	—	3	0.333
	IX	3	—	26	0.115
	XIII	8	1	52	0.154
Total		12	1	81	0.148
Grand total		51	6	303	0.168

Received March 29; revised June 7, 1971.

- ¹ Mukherjee, B. B., and Sinha, A. K., *Proc. US Nat. Acad. Sci.*, **51**, 252 (1964).
- ² Hamerton, J. L., Giannelli, F., Collins, F., Hallett, J., Fryer, A., McGuire, V. M., and Short, R. V., *Nature*, **222**, 1277 (1969).
- ³ Mukherjee, B. B., Mukherjee, A. B., and Mukherjee, A. B., *Nature*, **228**, 1321 (1970).
- ⁴ Hamerton, J. L., and Giannelli, F., *Nature*, **228**, 1323 (1970).
- ⁵ Cohen, M. M., and Rattazzi, M. C., *Proc. US Nat. Acad. Sci.*, **68**, 544 (1971).
- ⁶ Giannelli, F., and Hamerton, J. L., *Nature*, **232**, 315 (1971).
- ⁷ Cooper, J. E. K., *Texas Rep. Biol. Med.*, **28**, 29 (1970).

- ⁸ Trujillo, J. M., Walden, B., O'Neil, P., and Anstall, H. B., *Science*, **148**, 1603 (1965).
- ⁹ Lyon, M. F., *Ann. Rev. Genet.*, **2**, 31 (1968).
- ¹⁰ Lyon, M. F., *Phil. Trans. Roy. Soc.*, **B**, **259**, 41 (1970).
- ¹¹ Grüneberg, H., *J. Embryol. Exp. Morphol.*, **22**, 145 (1969).
- ¹² Sharman, G. B., *Nature*, **230**, 231 (1971).
- ¹³ Richardson, B. J., Czappon, A. B., and Sharman, G. B., *Nature*, **230**, 154 (1971).
- ¹⁴ Cooper, D. W., Vandenberg, J. L., Sharman, G. B., and Poole, W. E., *Nature*, **230**, 155 (1971).
- ¹⁵ Davidson, R. G., Nitowsky, H. M., and Childs, B., *Proc. US Nat. Acad. Sci.*, **50**, 481 (1963).
- ¹⁶ Linder, D., and Gartler, S. M., *Amer. J. Human Genet.*, **17**, 212 (1965).

Non-Random Late Replication of X Chromosomes in Mules and Hinnies

F. GIANNELLI

Paediatric Research Unit, Guy's Hospital Medical School, London SE1

J. L. HAMERTON

Department of Genetics, Children's Hospital, Winnipeg, and Departments of Paediatrics and Anatomy, University of Manitoba

Autoradiography of lymphocytes and fibroblasts has shed more light on the behaviour of X chromosomes in mules and hinnies.

THE process of DNA replication and genetic activity in X chromosomes of female mules and hinnies has been studied¹⁻⁶ without any agreement on the relative frequency with which the two parental X chromosomes (X^D and X^H) become late replicating and genetically inactive. Additional autoradiographic results on six female mules (*E. asinus* ♂ × *E. caballus* ♀) and eight female hinnies (*E. caballus* ♂ × *E. asinus* ♀) are presented here. One of the hinnies (No. 203) was bred in Cambridge⁵, three of the mules (Nos. 27, 33, 100), subject of a preliminary communication², were obtained from various sources in Great Britain, while the remainder of the hybrids were from Portugal.

Lymphocyte and fibroblast cultures were established by standard methods^{7,8}. Mixed lymphocyte cultures, using female donkey and horse blood were set up as controls, for the hybrid cultures. ³H-Tdr (specific activity 5 Ci/mmol, Amersham) in a concentration 0.3–0.5 µCi/ml. was added to the leucocyte cultures 3–4 h before collection and colcemid was added 1.5 h before termination of the culture. Fibroblast cultures were labelled with 0.3 µCi/ml. 6 h before termination and colcemid was added 3 h later. Slides were prepared by air drying, stained lightly with lacto-acetic orcein and suitably

spread metaphase plates were photographed. Autoradiographs were prepared by standard methods^{2,9}. The cells which had previously been photographed were relocated and examined for a differentially labelled X chromosome. (This was defined as a medium sized chromosome which had at least twice as many autoradiographic grains as any other chromosome of similar size. This definition was adopted because the donkey has a submetacentric autosome much larger than the X and with a large heterochromatic paracentric region which is often densely labelled in late S.) The morphology of the differentially labelled X chromosome (DLX) was then determined by examination of the photographs taken before autoradiography. The two X chromosomes in the female hybrids are quite different, one (X^D) being submetacentric, while the other (X^H) is metacentric^{1,2} (Fig. 1).

Twenty-one suitable metaphase plates from each of two hinnies and one mule (Nos. 100, 188, 203) were selected for quantitative autoradiographic studies, and karyotypes prepared. The total autoradiographic grains for each cell and for each of the five longest medium sized submetacentric and metacentric chromosomes were then counted. Grain counts were also made on samples of cells from the mixed leucocyte cultures, selected either at random or for the presence of a differentially labelled X chromosome.

The blood and skin cultures from the fourteen animals studied contained varying proportions of labelled cells and of cells with a differentially labelled X chromosome (Table 1). This did not influence the relative frequency of cells with a differentially labelled X^D or X^H . The relative frequencies differed significantly from 0.5 in every hybrid from which

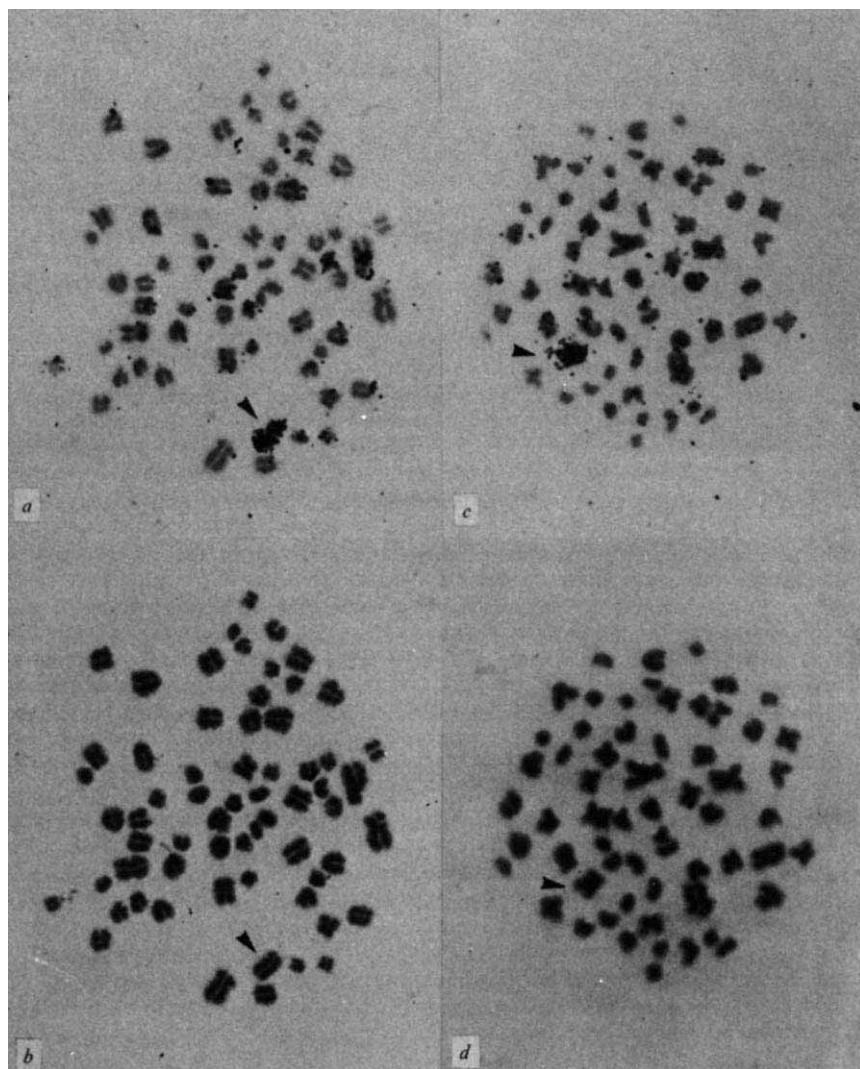
enough cells could be analysed and in each case there was an excess of DLX^D (Table 4). In the mixed leucocyte cultures, on the other hand, the number of horse cells which has a DLX was greater than the number of donkey cells with a DLX (Table 2).

The relative frequencies of DLX^D and DLX^H in cultures from individual mules or hinnies did not differ significantly from the overall frequencies for their own group (Tables 1 and 3). The hinnies behave in a similar fashion to the mules, but are possibly less homogeneous and seem to show a slightly higher frequency of DLX^H (Table 3).

the total cell grain counts was very similar in the horse and donkey cells. The covariance analysis (Table 5) showed that the grain counts over the X^H and X^D were very similar after allowance had been made for the labelling over the rest of the complement.

These results show that in both crosses irrespective of the direction of the cross, X^D is consistently more often differentially labelled than X^H . This differs from the findings of Mukherjee and his co-workers^{1,3} but is in broad agreement with the observation of Cohen and Rattazzi⁶.

Fig. 1 Two metaphase plates from the same hybrid, the first showing (a and b) a submetacentric chromosome (arrowed) and the second (c and d) a metacentric chromosome (arrowed) differentially labelled. These chromosomes presumably represent, in order, the donkey and horse-derived X chromosomes.



In the sample of twenty cells selected at random from a mule and each of two hinnies (Table 4) the grain counts over the most densely labelled medium sized submetacentric and metacentric chromosomes showed the same regression line on the total cell grain counts, but the former chromosome had clearly higher grain counts than the latter. If these chromosomes are taken to represent respectively the X^D and X^H chromosome, these data seem to agree with the qualitative observation that the X^D chromosome is more frequently differentially labelled. Furthermore, the proportion of X chromosome grains counted over X^D seems to be very similar in the mule and the two hinnies.

Finally, in the mixed leucocyte cultures the regression of the grains counted over the differentially labelled X chromosomes, or over the most densely labelled X -like chromosomes, on

Mukherjee and Sinha¹ used both longer labelling and colchicine times for blood cultures, while for skin cultures Mukherjee *et al.*³ used a shorter labelling time than ours. Their results, however, differ in the same direction in both culture systems from those reported here; this suggests that the discrepancy cannot be explained by a variation in labelling procedure.

In the present study the DLX was defined as the chromosome of compatible morphology which had at least twice as many grains as any other chromosome of a similar size. This is in agreement with the illustration of Mukherjee and Sinha¹ who did not discuss their criteria in detail, but is less strict than that used in their later article³ in which the DLX is defined as the chromosome with a grain count of at least 50% the total grain count over the whole complement.

In our opinion, this definition is too strict because of the difficulty of counting fifty grains or more over an X -like chromosome, which means that all cells with a total grain count of 100 or more over the remainder of the complement would have to be rejected. To see whether a more strict definition of DLX could affect the results presented here, however, all cells in which the grain counts over the DLX were not greatly in excess of twice that over any chromosome of similar size were rejected, leaving only those cells with a "very hot" DLX available for analysis. This procedure did

Data from the control cultures suggest that this is not so. The mixed lymphocyte cultures showed that the horse lymphocytes have a G_2 similar to that of the donkey and have a differentially labelled X chromosome at least as frequently. Admittedly a mixed lymphocyte culture is not a perfect control for cultures from hybrids in which each chromosome must be compared with the haploid sets from both parental species. But covariance analysis of grain counts over the DLX in the mixed lymphocyte cultures shows that, when account is taken of the labelling over the rest of the complement, DLX^D and

Table 1 Autoradiographic Data from Six Mules and Eight Hinnies

Animal No.	Tissue cultured	No. of cells scored	Total No. of labelled cells	No. of cells with a DLX Total	No. DLX^D	No. of cells with a "very hot" $DLX^\dagger$ Total	No. DLX^D
Mules							
27	Skin	70	65	14	13	8	8
33	Blood *	1,000	35	27	24	19	18
	Skin	105	77	45	43	33	33
100	Blood *	755	453	68	60	55	50
	Blood †	15	9	3	3	2	2
	Skin	45	40	8	8	8	8
143	Blood *	12	2	1	1	1	1
	Blood †	18	16	7	7	5	5
144	Blood *	22	13	8	7	7	6
	Blood †	142	124	33	28	28	24
145	Blood *	12	7	1	1	0	0
	Blood †	17	14	2	2	2	2
Totals	Blood *	1,801	510	105	93	82	75
	Blood †	192	163	45	40	37	33
	Skin	220	182	67	64	49	49
Hinnies							
138	Blood *	9	5	2	2	1	1
	Blood †	3	2	0	0	0	0
139	Blood *	27	12	6	6	5	5
187	Blood *	172	155	28	22	21	18
	Blood †	17	16	0	0	0	0
188	Blood *	168	120	13	11	8	8
	Blood †	23	22	0	0	0	0
189	Blood *	326	133	18	12	14	10
190	Blood *	152	127	12	8	9	6
	Blood †	37	32	1	1	0	0
191	Blood *	159	135	5	5	3	3
	Blood †	32	30	4	3	3	2
203	Blood *	165	105	23	18	17	13
	Blood †	131	121	211	17	19	16
Totals	Blood *	1,178	792	107	84	78	64
	Blood †	243	223	26	21	22	18

* 3 h incubation with $^3\text{H-Tdr}$. † 4 h incubation with $^3\text{H-Tdr}$.

† DLX with at least 2.2 times the grains counted over any other chromosome of similar size.

not alter the ratio of DLX^H to DLX^D (Table 1). It seems reasonable to conclude from this that the way in which the DLX is defined is unlikely to account for the differences observed. Could any peculiarity or intrinsic difference in the labelling of X^D and X^H , not associated with the process of facultative heterochromatinization of the X chromosome, for example, a higher rate of DNA synthesis in X^D than in X^H at some stage of late S, be responsible for the difference which we have observed between the frequency of differentially labelled X^D and X^H in the hybrids' cultures?

DLX^H label in a similar fashion during the part of S studied. This is confirmed by the similarity of the regression of grain counts over the presumptive X^D and X^H on total complement grain counts (TGC) in the three animals studied quantitatively (Table 4). Furthermore, an analysis of lymphocyte cultures from the hybrids shows that with an increase in labelling time the proportion of labelled cells increases and that of cells with a DLX decreases, while the relative frequency of DLX^D and DLX^H remains the same (Tables 1 and 6).

Finally, can these results be explained on the basis of an error in the identification of the X^D due to the presence of a

morphologically similar late replicating autosome which mimics the behaviour of the *DLX*? Our definition of the *DLX* precludes this explanation unless the *DLX^H* had less than half the grains counted over this autosome much more often than the *DLX^D*. This could occur either if the *DLX^H* usually had lower grain counts than the *DLX^D* or if it was in the differentially labelled state less often. The data presented do not

expression of an *X*-linked locus implies a definite relationship between these two phenomena; proof of such a relationship will be provided by autoradiographic studies on the clones discussed in the previous article⁵. These are in progress. A comparison of *X* chromosome behaviour and *Gd* expression in both the mule and the hinny suggests that neither the maternal cytoplasm nor the maternal environment is important in

Table 2 Autoradiographic Data from Mixed Lymphocyte Cultures

Cell type	No. of cells scored	No. of labelled cells	Cells with a <i>DLX</i> No. Proportion	Difference in proportions	s.e.	<i>P</i>
Horse	158	144	33 0.2291	0.1205	0.0515	0.05 > <i>P</i> > 0.025
Donkey	96	92	10 0.1086			

fit the first of these alternatives so that it seems reasonable to accept the second.

A further possibility which might account for our results is biased selection of cells or chromosome identification. To avoid this, the analysis of autoradiographs was conducted as independently as possible of the morphological chromosome analysis (a chromosome was classified as differentially labelled before analysis of the unlabelled photographs, and karyotypes were made by an individual who was unaware of the autoradiographic results). It is difficult, however, completely to exclude bias in an analysis of this type. The most serious cause of observer bias is the *a priori* expectations. In the study described here our intention was to investigate the relationship between differential labelling and genetic inactivity of one *X* chromosome, and it was expected that a 1:1 relationship would be found between the *DLX^H* and *DLX^D* in accordance with earlier findings¹. Preliminary observations on differential labelling in cells with three mules² showed this was not so and it was considered a tentative hypothesis that the reciprocal cross might show the reverse, namely a greater frequency of *DLX^H* than *DLX^D*. On both counts therefore the results reported here are contrary to *a priori* expectations and lead to the conclusion that *X^D* is significantly more often differentially labelled than *X^H* in both mules and hinnies.

These results must now be considered in relation to the Lyon hypothesis (LH). In contrast to the suggestion of others^{1,3} the mule may not provide a simple cytological test of random inactivation for reasons which have already been discussed⁴. These hybrids are, however, useful in studying the relationship between differential labelling of one *X* chromosome and genetic inactivation. Data on glucose-6-phosphate dehydrogenase (*Gd*)⁵ show that the frequency with which *Gd^D* is expressed in fibroblast clones derived from three hybrids is in almost exact inverse proportion to the frequency with which *X^D* is observed to be differentially labelled in both lymphocytes and fibroblasts. Furthermore, qualitative data on expression of *Gd^D* show that, with the exception of one animal, it is expressed far more weakly than *Gd^H* in all the hybrids studied¹⁰. Cohen and Rattazzi⁶ have reported a close correlation between the frequency of *DLX^D* and the expression of *Gd^D* in four further female mules. In each animal the *X^D* was more frequently late replicating and, correlated with this, *Gd^D* was the minor component. Hook and Brustman¹⁰ have studied *Gd* expression in organs from 37 female mules. They found that in most tissues (blood, pancreas, brain, cervical cord, kidney and parotid gland) *Gd^H* is preferentially expressed. In thyroid and lung there appeared to be random expression while in the liver *Gd^D* seemed to be preferentially expressed.

The excellent correlation observed in our studies and those of Cohen and Rattazzi⁶ between differential labelling and the

determining which *X* chromosome becomes genetically inactive and differentially labelled in the female hybrids or in determining which cell type proliferates preferentially

Table 3 Observed Proportion of Cells with an *X^D* Differentially Labelled and their 95% Confidence Limits

Type	Hybrids No.	Tissue	Proportion <i>DLX^D</i>	95% Confidence limits
Mules	27	S	0.928	0.661–0.998
	33	B	0.889	0.708–0.976
		S	0.955	0.848–0.995
	100	B	0.887	0.790–0.950
		S	1.00	0.631–1.00
	143	B	1.00	0.631–1.00
	144	B	0.854	0.708–0.944
	145	B	1.00	0.292–1.00
	Total	B	0.887	0.824–0.932
		S	0.955	0.875–0.991
Hinnies	138	B	1.00	0.158–1.00
	139	B	1.00	0.541–1.00
	187	B	0.786	0.590–0.917
	188	B	0.846	0.545–0.981
	189	B	0.667	0.410–0.867
	190	B	0.692	0.386–0.909
	191	B	0.889	0.517–0.997
	203	B	0.795	0.647–0.902
Total	Total	B	0.789	0.747–0.885
		B	0.841	0.801–0.853

S, Skin fibroblasts; B, lymphocytes.

The significant excess of cells with an inactive and differentially labelled *X^D* is most easily explained by a difference between the two *X* chromosomes either in respect to the mechanism of "induction" of facultative heterochromatin or to the increased fitness which an active *X^H* confers on the hybrid cells resulting in selection in their favour. There are at least two alternatives which might account for a difference between *X^D* and *X^H* in regard to the mechanism of heterochromatinization; first, that *X^D* is simply more prone to become heterochromatic, or, second, that heterochromatinization commences earlier in the donkey than in the horse and that this is retained in the hybrids and so leads to an earlier involvement of *X^D* compared with *X^H*. The studies reported in the previous article⁵ on fibroblast clones suggest *in vitro* cell selection in

Table 4 Comparisons between the Grain Counts over the Most Densely Labelled X^D and X^H -like Chromosomes ($?X^D$, $?X^H$) in a Random Sample of Cells from Mule 100 and the Hinnies 188 and 203

Hybrid No.	No. of cells analysed	Comparison of mean grain counts over $?X^D$ and $?X^H$	Comparison of the regression coefficients of the logarithm of $?X^D$ and $?X^H$ grain counts on TGC *	Comparison of the contribution (D) of $?X^D$ to the grains counted over the presumptive X chromosomes in the mule and the two hinnies
100	21	$\bar{Y}_1 = 12.85$ $\bar{Y}_2 = 9.00$ $\bar{Y}_1 - \bar{Y}_2 = 3.85$ $s.e.(\bar{Y}_1 - \bar{Y}_2) = 1.35$ $P < 0.02$	$b_1 = 0.000956$ $b_2 = 0.001864$ $b_1 - b_2 = -0.000908$ $s.e.(b_1 - b_2) = 0.000869$ $P > 0.3$	$D_m = 0.5882$
188	20	$\bar{Y}_1 = 8.40$ $\bar{Y}_2 = 6.10$ $\bar{Y}_1 - \bar{Y}_2 = 2.30$ $s.e.(\bar{Y}_1 - \bar{Y}_2) = 0.964$ $P < 0.05$	$b_1 = 0.00209$ $b_2 = 0.00199$ $b_1 - b_2 = 0.00010$ $s.e.(b_1 - b_2) = 0.00077$ $P > 0.9$	$D_h = 0.5892$ $D_m - D_h = -0.0010$
203	18	$\bar{Y}_1 = 15.61$ $\bar{Y}_2 = 10.61$ $\bar{Y}_1 - \bar{Y}_2 = 5.00$ $s.e.(\bar{Y}_1 - \bar{Y}_2) = 2.25$ $P < 0.05$	$b_1 = 0.00102$ $b_2 = 0.00205$ $b_1 - b_2 = -0.00103$ $s.e.(b_1 - b_2) = 0.000613$ $P > 0.1$	$s.e.(D_m - D_h) = 0.023$ $P > 0.8$

* Scatter diagrams have shown that the regressions of the logarithms of the $?X$ chromosomes grain counts on the total complement grain counts (TGC) are linear.

1, $?X^D$; 2, X^H ; m, mule; h, hinny.

favour of cells with an active X^H ; studies by Hook and Brustman¹⁰ suggest that *in vivo* cell selection occurs in different organs.

Our finding of one animal (No. 192)⁵ with a preponderance of Gd^D , on which unfortunately autoradiographic studies were not possible, suggests that there may be inter-animal variation in the expression of Gd . Inter-animal variation could perhaps account also for the discrepancy between our autoradiographic results and those of Mukherjee and his colleagues^{1,3}.

Table 5 Comparison by Covariance Analysis of the Grain Counts over Differentially Labelled X Chromosomes (A) or the Most Densely Labelled X -like Chromosomes (B) in Horse-Donkey Mixed Blood Cultures

Analysis	A chromosome		B chromosome	
	X^D	X^H	$?X^D$	$?X^H$
No. cells analysed	10	33	20	20
Mean grain counts	$\bar{Y}_1 = 30.21$	$\bar{Y}_2 = 26.00$	$\bar{Y}_1 = 25.65$	$\bar{Y}_2 = 19.95$
Adjusted mean	$\bar{Y}_1 = 29.55$	$\bar{Y}_2 = 28.17$	$\bar{Y}_1 = 22.90$	$\bar{Y}_2 = 22.70$
Difference	$\bar{Y}_1 - \bar{Y}_2 = 1.38$		$\bar{Y}_1 - \bar{Y}_2 = 0.20$	
s.e. of difference	1.88		2.55	
Probability	$0.5 > P > 0.4$		$0.95 > P > 0.90$	

Scatter diagrams were made before these analyses. These have shown a linear relationship between the presumptive X chromosome grain counts and the counts over the rest of the complement. The variance of Y was fairly constant over the range of total grain counts used and this was similar in the two samples compared. The regression coefficients of the individual samples used in this comparison were significantly different from zero, but did not differ significantly from each other.

Our data and those of Cohen and Rattazzi⁶, however, clearly show that X^D tends to be more often heterochromatic than X^H in the tissues of female horse-donkey hybrids which have been available for study.

We thank Drs R. V. Short, W. R. Allen and M. J. Freire, with his colleagues in Portugal, for samples of material. Collection of Portuguese samples was supported by grants to J. L. H.

Table 6 Analysis of the Tendency for the Proportion of DLX^D to Vary according to the Proportion of Labelled Cells in Blood Cultures of Mules and Hinnies

Type of hybrid	Regression coefficient	SS due to regression	χ^2	P
Mules	-0.5341	352.4	0.429	> 0.5
Hinnies	-1.033	1,531	1.86	> 0.1
All *	-1.189	3,777	4.6	< 0.05

This analysis was conducted using the angular transformation method¹¹.

* Since the proportion of DLX^D in the hinnies is lower than that of mules and their cell samples contribute chiefly to the class with a relatively high proportion of labelled cells the data regarding both types of hybrid together are not very meaningful.

from the Wellcome Trust. During this work J. L. H. and F. G. were supported by the Spastics Society. J. L. H. also acknowledges support from ARC and the Canadian Medical Research Council. We also thank Mrs F. F. Collins, Mrs J. Hallett, Miss A. Fryer, Miss V. M. McGuire and Mr L. Grixti for technical assistance.

Received May 7, 1971.

- Mukherjee, B. B., and Sinha, A. K., *Proc. US Nat. Acad. Sci.*, **51**, 252 (1964).
- Hamerton, J. L., Giannelli, F., Collins, F., Hallett, J., Fryer, A., McGuire, V. M., and Short, R. V., *Nature*, **222**, 1277 (1969).
- Mukherjee, B. B., Mukherjee, A. B., and Mukherjee, A. B., *Nature*, **228**, 1321 (1970).
- Hamerton, J. L., and Giannelli, F., *Nature*, **228**, 1322 (1970).
- Hamerton, J. L., Richardson, B. J., Gee, P. A., Allen, W. R., and Short, R. V., *Nature*, **232**, 312 (1971).
- Cohen, M. M., and Rattazzi, M. C., *Proc. US Nat. Acad. Sci.*, **68**, 544 (1971).
- Hsu, T. C., and Kellogg, jun., D. S., *J. Nat. Cancer Inst.*, **25**, 221 (1960).
- Moorhead, P. S., Howell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A., *Exp. Cell Res.*, **20**, 613 (1960).
- Giannelli, F., *Human Chromosomes DNA Synthesis, Monographs in Human Genetics*, **5** (edit. by Beckman, L., and Hauge, M.) (Karger, Basel and New York, 1970).
- Hook, E. B., and Brustman, L. D., *Genetics*, **64**, 2, Part 2 (abstract, 1970).
- Fisher, R. A., and Yates, F., *Statistical Tables for Biological, Agricultural and Medical Research*, fifth ed. (Oliver and Boyd, Edinburgh, 1957).

LETTERS TO NATURE

PHYSICAL SCIENCES

Ivory Coast Microtektites: Fission Track Age and Geomagnetic Reversals

In this article we report our measurements of the age of Ivory Coast microtektites, and discuss the association of microtektites with geomagnetic reversals. Microtektites are small glassy particles (<1 mm) found in sediment cores from the oceans adjacent to the Australasian and Ivory Coast tektite fields¹. They resemble tektites in outward form and composition. Comparatively few microtektites have been recovered so far, but Cassidy *et al.*² believe that the total mass of Australasian microtektites may be at least 10^{10} kg, which is 10^5 times larger than the estimated mass of the tektites.

Our Ivory Coast microtektites were kindly given to us by Dr B. P. Glass, and we have used the fission track method of dating³. In this method the known rate of spontaneous fission of ^{238}U (we have used a fission decay constant of $8.42 \times 10^{-17} \text{ yr}^{-1}$) allows the age of the material to be determined after the uranium content of the material has been found by exposing

the material to a known dose of thermal neutrons. Then the age in years of the sample, A , is given by⁴

$$A = (\rho_n / \rho_i) 5.01 \times 10^{-8} F \quad (1)$$

where ρ_n and ρ_i are the natural and induced etch pits per unit area, and F is the time-integrated flux of neutrons (usually a few times 10^{15} thermal neutrons cm^{-2}).

To count as many natural fission tracks as possible in our limited sample, each microtektite was mounted individually in epoxy resin on a plastic backing, and polished to uncover successive layers for etching and examination under the microscope. The etchant was hydrofluoric acid (40% by volume) used for 30 s at 21° C. The initial diameters of the microtektites were ~150–400 μm , and each etching and polishing (using 0–1 μm diamond powder on a microcloth) removed a layer about 20 μm thick. The last few layers were usually saved for irradiation by thermal neutrons, and the same counting procedure was used for the irradiated samples.

Fig. 1 shows typical photomicrographs of natural and induced etch pits, and a poorly polished section including several spurious etch pits is shown for comparison. Altogether 26 microtektites were examined, some in greater detail than

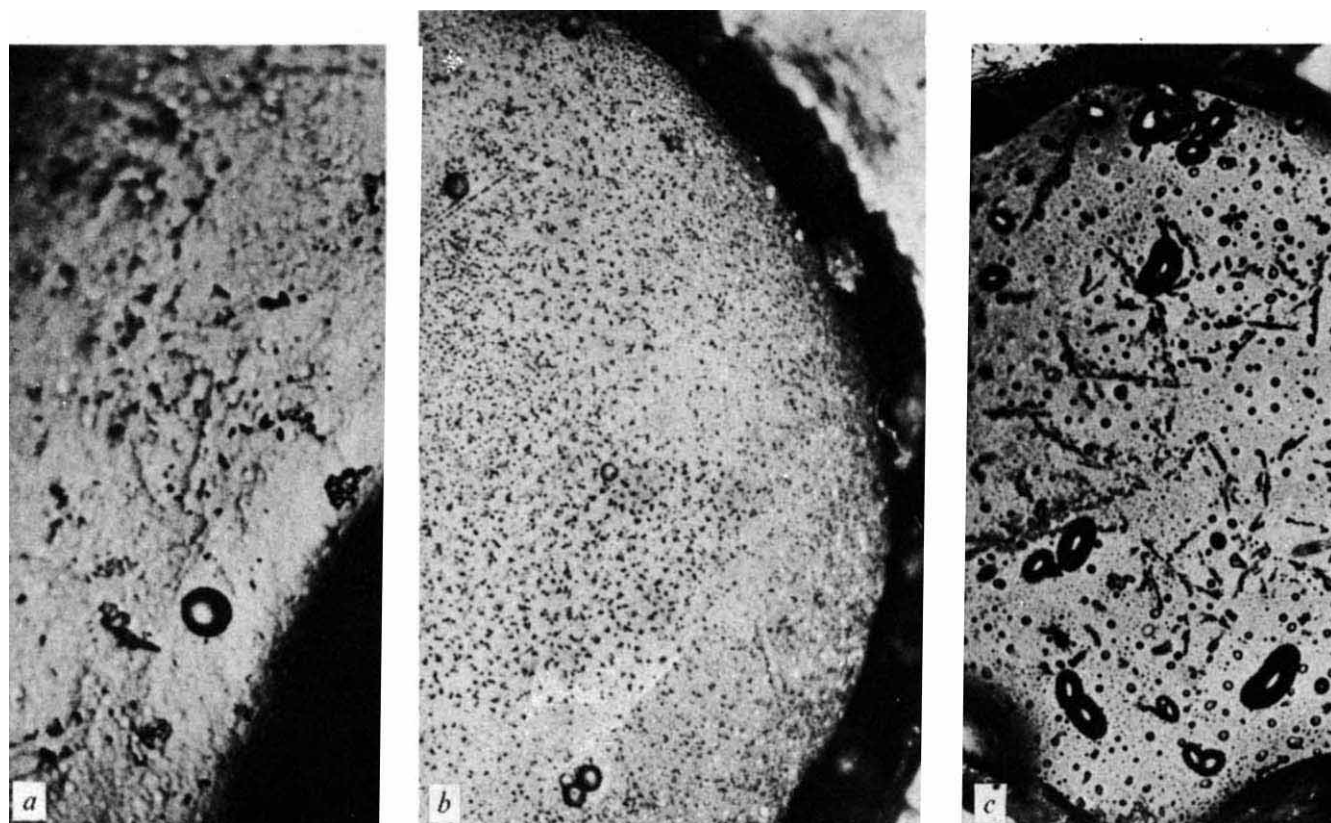


Fig. 1 Photomicrographs of fission tracks in Ivory Coast microtektites etched for 30 s in 40 volumes % HF at 21° C. The figure shows etch pits of (a) a spontaneous fission track and (b) induced fission tracks after irradiation with 3.22×10^{15} thermal neutrons cm^{-2} . In c most of the (kidney shaped) etch pits are of spurious origin, resulting from bad polishing. All photographs were taken with a Zeiss photomicroscope, using epi-illumination. (a and c, $\times 160$; b, $\times 48$.)

Table 1 Ages of Tektites and Microtektites from the Australasian and Ivory Coast Strewn Fields

Strewn field	Ages of tektites (m.y.)		Ages of microtektites (m.y.)		Deposition ages
	K/Ar ages	Fission track ages	Fission track ages Gentner <i>et al.</i> ⁵	Present work	
Ivory Coast	1.15 ±0.15*	1.01 ±0.10* 0.86 ±0.06¶	1.09 ±0.20†	0.88 ±0.13‡	~0.8§ ~1.0
Australasian	0.72 ±0.10*	0.70 ±0.10* 0.68 ±0.05¶	0.71 ±0.10	—	~0.7§

* Gentner *et al.*⁶

† Based on forty microtektites from the Atlantic Ocean core K9-56 (3° 22.8' N lat., 15° 37.2' W long., 2,563 fathoms depth).

‡ Based on twenty-six microtektites from core K9-56. Of the eighteen microtektites which could be successfully analysed (yielding fifty-seven natural tracks), thirteen were irradiated in a reactor for age and U content determinations.

§ Glass⁷.¶ Determined by us. The Ivory Coast tektite age is based on only one specimen (from Ouellet) kindly given to one of us (S. A. D.) by Professor F. Kraut, director, Museum of Natural History, Paris. A total of 1,073 natural tracks was observed over an area of 2.65 cm².

others; 57 natural tracks were found (in 18 microtektites) over an area of 40.89 mm², and 3,627 induced tracks over an area of 8.40 mm².

Table 1 summarizes our results and those of Gentner *et al.*⁵, based on the fission track method to date tektites and microtektites from the Australasian and Ivory Coast strewn fields. K-Ar ages of the tektites are also shown, but microtektites are too small for this method. Our ages for the Ivory Coast tektites and microtektites agree among themselves, although they are somewhat lower than those reported by other workers (except for the deposition age⁷ of ~0.8 to 1.0 m.y. for the microtektites). The errors shown in Table 1 for our fission track ages are made up of statistical (Poisson) errors, uncertainties in the neutron dose (±5%) and, for the microtektite measurements, uncertainties in the uranium content of the samples which could not be irradiated.

It has already been pointed out that the Australasian microtektites occur within a zone ~30–50 cm thick associated in time with the last geomagnetic reversal^{2,5,8}. In other words, the Australasian microtektites appear to coincide in age with the boundary between the present normal epoch (Brunhes) and the preceding reversed geomagnetic epoch (Matuyama), which is believed to have occurred about 0.7 m.y. ago according to magnetic measurements of lavas and deep-sea sediments^{9–11}. There have been four geomagnetic epochs of alternating polarity over the past 3.6 m.y., each lasting ~10⁶ yr, interspersed with shorter events (<0.2 m.y.) when the polarity was briefly antiparallel to that of the epoch. We believe that it may be possible to associate the Ivory Coast microtektites with the last such event (Jaramillo) which occurred about 0.9 m.y. ago^{12,13}. Ivory Coast microtektites have been found so far in two cores, V19–297 (2° 37' N lat., 12° 00' W long.) and K9–56 (3° 23' N lat., 15° 37' W long.). The microtektites in V19–297 are concentrated⁵ in a thin layer about 15 cm thick that occurs about 40 cm below the Matuyama–Brunhes boundary. The Jaramillo event has been lost in this core, but palaeontological stratigraphy suggests¹⁴ that it coincides with the fall of the microtektites. Our microtektites come from the K9–56 core, and our age of 0.88 ± 0.13 m.y. (as well as the weighted mean of our measurements and those of Gentner *et al.*, 0.94 ± 0.10 m.y.) is in close agreement with the estimates of the age of the Jaramillo event, which range from 0.85 to 0.97 m.y. Unlike the stratigraphic determinations of age in deep-sea sediments (whether palaeomagnetic or palaeontological) which may have

been affected by bottom currents and mud-burrowing organisms the fission track determinations are absolute. The age of the Ivory Coast tektite found by us, 0.86 ± 0.06 m.y., is also in good agreement with the date of the Jaramillo event.

Glass and co-workers^{2,8} have already suggested that the phenomenon responsible for the Australasian field may also have caused the Matuyama–Brunhes reversal, and similar arguments can be put forward to account for the Jaramillo event. These hypotheses of course do not say whether the mass of microtektites and tektites came from outside the Earth, or whether a comet or meteorite struck the Earth and scooped out material which then fell back to the Earth as a spray of tektites and microtektites. But it seems plausible from what is known of the cause of the geomagnetic field, and its instability, that such comparatively small disturbances could reverse the field direction.

In this context we wish to suggest a possible explanation of the sudden changes in marine fauna which seem to be associated with geomagnetic reversals. Some marine microorganisms become extinct, and others suddenly appear, in sediments at reversal levels¹⁵. If tektites are produced by cometary impacts, as Urey has suggested, then the gases such as frozen ammonia and methane contained in cometary nuclei might have appreciable ecological effects when rapidly introduced into the atmosphere and oceans. Some organisms thrive on methane, for example, while others do not; ammonia, which would dissolve rapidly in sea water, would have similar effects. Large explosions of methane–air mixtures, sparked by atmospheric lightning, may also have occurred. There needs to be a quantitative examination of whether cometary impact could cause reversals and produce faunal changes over large areas of the globe.

The cometary hypothesis also avoids the difficulties which have appeared in the lunar-origin hypothesis of tektites and microtektites now that lunar samples are available. Although minute glassy particles that look like microtektites have been found on the Moon^{16,17}, the chemical compositions are not similar¹⁸. The resemblance of the exceptional lunar sample 12013 (ref. 17) with two untypical Java tektites^{18,19} is not firm evidence of affinity.

Finally, we wish to describe in more detail some of the precautions taken in our age measurements of the Ivory Coast microtektites. In determining the age of a material by the fission track method it is important to ensure that no thermal effect that would lower the measured age has occurred in nature. Heating results in the fading of tracks and in shrinkage of the etch-pit diameters of the surviving tracks^{20,21}; it can be corrected for by comparing the diameters of the etch pits for natural and freshly induced tracks. These correction factors are obtained by a series of isothermal annealing experiments, and Fig. 2 shows a comparison of the natural and induced fission etch-pit diameters of Ivory Coast microtektites. The *t* test shows no significant difference; so the observed age may be regarded as the true age. The same applies to the Ivory Coast tektite (natural diameter: 4.65 ± 1.24 μm; induced diameter: 4.67 ± 1.09 μm).

The uranium content in p.p.m. by weight is given by

$$C = 9.445 \times 10^7 \rho_1 / (D \cdot R_{ap} \cos^2 \theta_c \cdot g \cdot f(t) \cdot F) \quad (2)$$

where *D* is the density of the material (g cm⁻³), *R_{ap}* is the apparent range of an average fission fragment, *θ_c* is known as the critical angle of etching, *g* is a geometrical factor and *f(t)* the prolonged etching factor for the material (our unpublished work). *D · R_{ap}* is taken to be the same as found for a reference glass (U-2) with a known amount of uranium (that is, 2.41 g cm⁻³ × 7.6 μm), and *g* = 1 (for 4 π geometry, the contribution of fission fragments from both sides of each etched surface being taken into account). *ρ₂* and *F* are as in equation (1).

The critical angle³ is defined as the minimum track inclination with the surface that leads to successful etching. We have determined this angle for microtektites using a novel com-

parison method. A ^{252}Cf spontaneous-fission source was used to bombard first an indochinite and then a microtektite for equal lengths of time under identical ("2 π ") geometry. The tektite and the microtektite were then etched in identical conditions (30 s at 21° C in 40 volumes % HF). The number of fission-fragment etch pits per unit area can be shown to be in the ratio $(1 - \sin\theta_{c,1})/(1 - \sin\theta_{c,2})$, where the subscripts 1 and 2 refer to the two materials being compared. The critical angle for the microtektite is found to be 24° 13', which is in good agreement with that for the indochinite (that is, 25° 21', determined in a similar manner by comparison with a glass slide of known critical angle). The error in each case is $\pm 30'$. This agreement in itself is a confirmation of the chemical similarity of tektites and microtektites. (The critical angle for the soda-lime glass slide, in contrast, is 35° 30'.)

The prolonged etching factor $f(t)$ results from the fact that, as the etching process progresses and new surfaces are revealed, fresh fission tracks are continually encountered and developed. This growth in numbers is not compensated by a corresponding obliteration of tracks which had begun to be developed earlier and whose actual end-points may have been reached in the meantime, for they continue to yield recognizable, although progressively shallower (less acute) etch pits. The function $f(t)$ is defined as the ratio ρ_t/ρ_0 , where ρ_t is the track density observed on etching the sample for time t , and ρ_0 is the value for zero etching-time (the density of "etchable tracks" in any surface), obtained by extrapolating the experimental growth curve backwards to $t=0$. Neglecting the factor $f(t)$ in equation (2) would result in an overestimate of the true value of uranium content (whereas in the case of age determination this factor cancels out if both the natural and the irradiated samples are etched in identical conditions).

Fig. 3 shows the growth of observed etch-pit densities with the duration of etching for an irradiated microtektite, an indochinite, and the (soda-lime) reference glass U-2 (all etched in 40 volumes % HF at 21° C— $f(t)$ is temperature-dependent). The value of $f(t)$ for the microtektite is found to be 1.16 for an etching time $t=30$ s. Again, the fact that the growth curves for microtektites and tektites are nearly parallel (whereas that for soda-lime glass is much steeper) demonstrates their kinship.

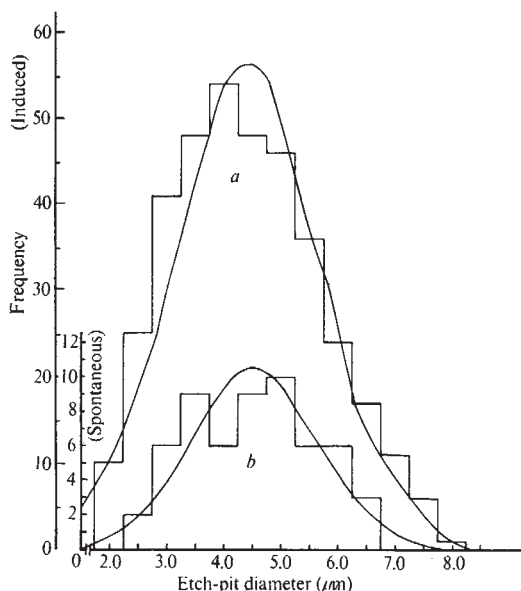


Fig. 2 Comparison of the etch-pit diameters of spontaneous and induced fission tracks in Ivory Coast microtektites etched for 30 s in 40 volumes % HF at 21° C. In the case of elliptical pits, the major axis has been taken instead of the diameter. A Gaussian has been fitted to each histogram. The absence of a significant difference between the two distributions indicates that no thermal lowering of age has occurred in the natural microtektites. a, Induced fission (4.41 ± 1.30 μm , 367 tracks; b, spontaneous fission (4.52 ± 1.09 μm , 57 tracks).

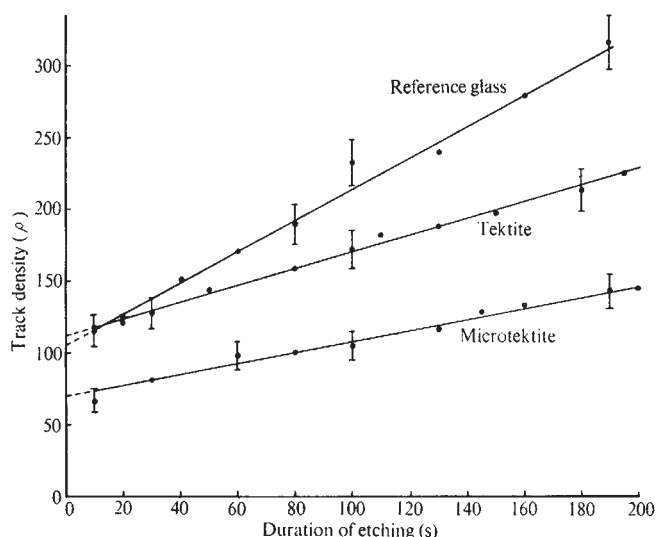


Fig. 3 Prolonged etching factor $f(t)$. The diagram shows the cumulative growth in the number of fission tracks (observed over an arbitrarily chosen area of 0.15 mm^2 in each case) as a function of the duration of etching. Irradiated sections of a microtektite, a tektite and (soda-lime) reference glass have been etched in identical conditions (30 s in 40 volumes % HF at 21° C). The value of $f(t)$ is defined as the ratio of the track density ρ at time t to that at time $t=0$ (see text for discussion); it is found to be 1.16 for the microtektite, 1.15 for the tektite, and 1.41 for reference glass (at $t=30$ s in the etching conditions used). The representative error bars shown indicate (Poisson) standard deviations of actual numbers observed.

The mean value of uranium content, based on thirteen irradiated microtektites, is found to be 0.43 p.p.m. by weight (corresponding to 0.81×10^4 tracks cm^{-2} induced by a fluence of 1×10^{15} neutrons cm^{-2}), with a range of 0.37 to 0.51 p.p.m. between individual samples. This may be compared with an average of 0.23 p.p.m. (and a range of 0.12 to 0.48 p.p.m.) calculated from the results of Gentner *et al.*⁵ Incidentally, our determination of the Ivory Coast tektite uranium content, that is, 1.3 p.p.m., compares well with the value reported by Rybach and Adams²², namely, 1.10 p.p.m. (mean of twenty samples).

In conclusion, the fact that the ages of the Ivory Coast and Australasian microtektites and tektites seem to coincide with the dates of the last two geomagnetic reversals raises the important question of a causal relationship. If a cometary impact with the Earth resulted in the production of tektites and microtektites, and in the reversal of the geomagnetic field, an interesting corollary is the possible effect of the released gases on the ecological balance on land and sea areas.

We thank the Global Ocean Floor Analysis and Research Group (USNOO), the Science Research Council and the staff of HERALD reactor at AWRE, Aldermaston, and Dr D. Storzer, Dr G. A. Wagner, Professors Sir E. C. Bullard, H. C. Urey and J. H. Fremlin, Dr M. H. Dodson and Dr C. R. Sladden. One of us (H. A. K.) thanks the Pakistan AEC and the Colombo Plan authorities for support.

S. A. DURRANI
H. A. KHAN

Department of Physics,
University of Birmingham,
Birmingham B15 2TT

Received May 10; revised June 25, 1971.

¹ Glass, B., *Nature*, **214**, 372 (1967).

² Cassidy, W. A., Glass, B., and Heezen, B. C., *J. Geophys. Res.*, **74**, 1008 (1969).

³ Fleischer, R. L., Price, P. B., and Walker, R. M., *Ann. Rev. Nucl. Sci.*, **15**, 1 (1965).

⁴ Durrani, S. A., and Hancock, D. A., *Earth Planet. Sci. Lett.*, **8**, 157 (1970).

- ⁵ Gentner, W., Glass, B. P., Storzer, D., and Wagner, G. A., *Science*, **168**, 359 (1970).
- ⁶ Gentner, W., Storzer, D., and Wagner, G. A., *Naturwissenschaften*, **56**, 255 (1969).
- ⁷ Glass, B. P., *Geochim. Cosmochim. Acta*, **33**, 1135 (1969).
- ⁸ Glass, B., and Heezen, B. C., *Nature*, **214**, 372 (1967).
- ⁹ Bullard, E. C., *Phil. Trans. Roy. Soc.*, **A263**, 481 (1968).
- ¹⁰ Cox, A., Doell, R. R., and Dalrymple, G. B., *Nature*, **198**, 1049 (1963).
- ¹¹ Ninkovich, D., Opdyke, N., Heezen, B. C., and Foster, J. H., *Earth Planet. Sci. Lett.*, **1**, 476 (1966).
- ¹² Cox, A., *Science*, **163**, 237 (1969).
- ¹³ Cox, A., and Dalrymple, G. B., *J. Geophys. Res.*, **72**, 2603 (1967).
- ¹⁴ Ericson, D. B., and Wollin, G., *Science*, **162**, 1227 (1968).
- ¹⁵ Ericson, D. B., Ewing, M., and Wollin, G., *Science*, **139**, 727 (1963).
- ¹⁶ Lunar Sample Preliminary Examination Team, *Science*, **165**, 1211 (1969).
- ¹⁷ Lunar Sample Preliminary Examination Team, *Science*, **167**, 1325 (1970).
- ¹⁸ Durrani, S. A., *Phys. Earth Planet. Interiors*, **4**, 251 (1971).
- ¹⁹ O'Keefe, J. A., *Science*, **168**, 1209 (1970).
- ²⁰ Storzer, D., and Wagner, G. A., *Earth Planet. Sci. Lett.*, **5**, 463 (1969).
- ²¹ Durrani, S. A., and Khan, H. A., *Earth Planet. Sci. Lett.*, **9**, 431 (1970).
- ²² Rybach, L., and Adams, J. A. S., *Geochim. Cosmochim. Acta*, **33**, 1101 (1969).

Measurements of the Gegenschein from Space

THE zodiacal light, which is produced by light scattered from small interplanetary particles, gradually declines in brightness with increasing angle of elongation, ϵ , from the Sun. As the anti-solar point ($\epsilon = 180^\circ$) is approached, the surface brightness again increases slightly to produce the counter-glow or gegenschein. This diffuse feature can be best observed against the sky background when it lies outside the region of the Milky Way. Fig. 1 depicts the sky background brightness at the anti-solar point as a function of time throughout the year. The integrated light from stars predominates during the two Milky Way traverses. Observations of the gegenschein are made best away from the Milky Way in February, March and April, or in August, September and October.

Ground-based observations are weakened by atmospheric extinction and diluted by the upper atmosphere airglow and light scattered in the Earth's lower atmosphere. Measurements of the gegenschein from above the Earth's atmosphere avoid the difficulties of these atmospheric effects. Accordingly, an

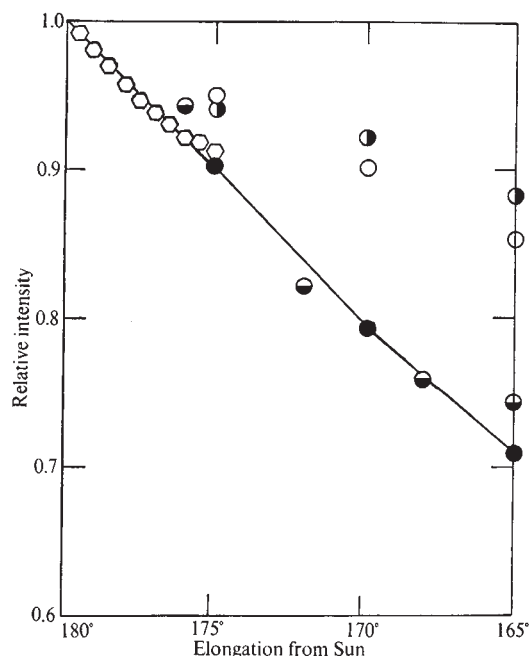


Fig. 2 Relative intensity distribution in the gegenschein. Comparison of the brightness distribution of the gegenschein as measured by ground observers with OSO-6 measurements (●) at 5000 Å. ○, ref. 2; ○, ref. 4; ◐, ref. 3; ●, ref. 1.

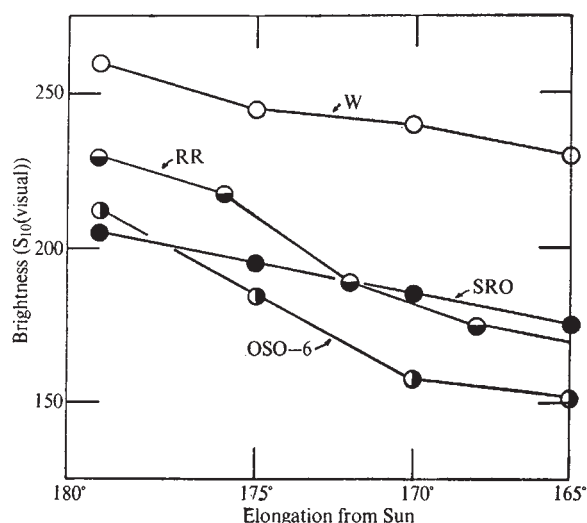


Fig. 3 Absolute gegenschein brightness comparison of the absolute surface brightness in the gegenschein from OSO-6 observations and ground-based observers (W: ref. 3; RR: ref. 1; SRO: ref. 4). Preliminary calibration of the space observations.

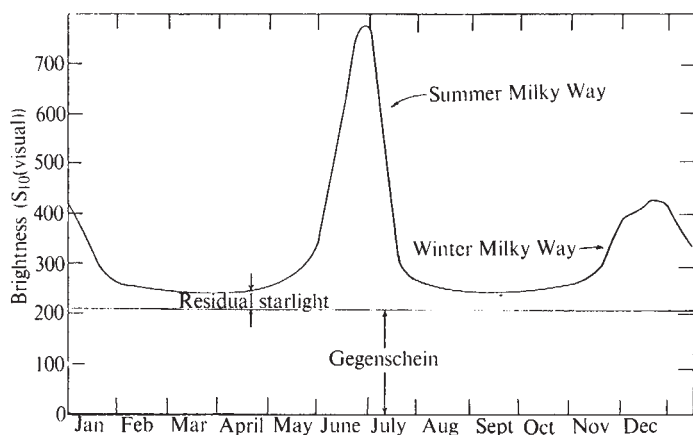


Fig. 1 Sky brightness at the anti-solar point as a function of time as it would appear to an observer above the Earth's atmosphere. Ordinates give surface brightness in units of tenth magnitude stars per square degree, S_{10} (visual). The contributions of the gegenschein are assumed constant, but the starlight (plus galactic light) contributions exhibit maxima during Milky Way traverses.

experiment was designed for the wheel of an orbiting solar observatory (OSO-6) to measure the brightness and polarization of the sky. The photometer-polarimeter, which was built by Ball Brothers Research Corporation includes a Sun-shielded objective, a collimator, quartz rotators, a quarter-wave plate, a double-cut prism, interference filters and a field lens, all bringing the light beam through a 2° diameter field stop to an EMR 641 E-type photomultiplier. Although the instrument was designed to measure polarization, we are concerned here only with photometric data at 5000 Å. The equipment is operable only during satellite daytime and scattered light enters the photometer when the Sun (or the sunlit Earth) is at an elongation 90° or less with respect to the line of sight. The gegenschein region, however, is free of photometric contamination from this effect.

Comparison of measurements made towards the same point in the celestial sphere at two different points in the orbit, plus comparison of the space-based and ground-based observations of the Milky Way, indicate that the instrument is operating well and yielding trustworthy information.

In Fig. 2 we compare the relative brightness of space measurements of the gegenschein within 15° of the anti-Sun position with some corresponding ground-based results. The present space measurements are in accord with the data of Roach and Rees¹ and the recent studies of Roosen² in suggesting a fairly steep photometric gradient from the anti-Sun position. Weinberg³ and Smith, Roach and Owen⁴ find a less steep gradient. Fig. 3 shows a comparison of the absolute brightness of the gegenschein as measured from space in comparison with several published ground-based observations. The space observations are based on a preliminary absolute calibration.

As further material becomes available we hope to extend the discussion to $\pm 25^\circ$ from the anti-solar position and improve the absolute calibration. Also, the photometry will be extended to other colours (4000 Å and 6100 Å) and the polarization data evaluated. The exact variation of the polarization at elongations between 150° and 180° is of special interest in assessing the nature of the scattering particles.

We thank R. Wilson, H. Zimmerman, and Wm. Dagle of Ball Brothers Research Corporation for the assembly of the polarimeter-photometer. This work was supported by a NASA grant to Rutgers University.

A. ROUY*
B. CARROLL

Rutgers University,
Newark, New Jersey

L. H. ALLER

University of California,
Los Angeles

Battelle Northwest Laboratories
and Rutgers University

Received June 21, 1971.

* Deceased March 2, 1970.

¹ Roach, F. E., and Rees, M. H., *Airglow and Aurorae* (Pergamon, London, 1956).

² Roosen, R. G., *Icarus*, **13**, 184 (1970).

³ Weinberg, J. L., *Ann. d'Astrophys.*, **27**, 218 (1964).

⁴ Smith, L., Roach, F. E., and Owen, R. W., *Planet. Space Sci.*, **13**, 207 (1965).

Martian Blue Haze Clearings and Flash Phenomena

THE relative lack of contrast in the blue ($\lambda < 0.44 \mu\text{m}$) in examinations of the surface markings of Mars has long been known, but occasionally and unpredictably, detailed blue structure can be seen briefly during blue haze clearings. There is some controversy about these phenomena^{1,2}, although in our opinion they are well established. A blue obscuring haze may be attributed to scattering of light by suspended particles³⁻⁷ of diameter less than $0.4 \mu\text{m}$, in the martian atmosphere so that blue haze clearings would result whenever meteorological conditions caused a partial precipitation of particles, but the mechanisms for the removal remain as obscure. We propose a simple meteorological mechanism which is consistent with available data from martian oppositions and flash observations.

There is evidence⁸ of significant water on Mars. Although the occurrence of liquid water would be a transitory, non-

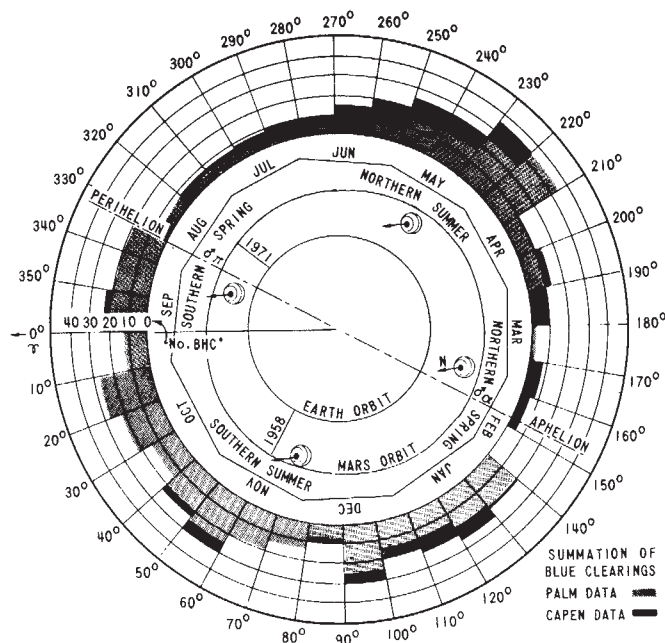


Fig. 1 Histogram chart of martian blue haze clearings (BHC), plotted against martian heliocentric longitude. All seasons shown are martian but months are terrestrial. *The scale shows the number of BHC per 10° sector. The Palm data are opposition data; Capen data are general data for the period October 1966 through March 1968. Earth months correlate with opposition (Palm) data. Arrows on Mars indicate approximate North Pole orientation. It must be kept in mind that this histogram is, in part, a function of the positions of the terminal points of the interval over which one averages in representing the discrete histogram representation of what is really a smooth curve.

equilibrium possibility, Shorn⁸ speculates that the triple point pressure may be exceeded occasionally. Fine martian atmospheric dust is assumed to influence the haze. Essentially, our clearing mechanism is the removal of available atmospheric dust by the condensation of water vapour on dust nuclei during highest humidity, resulting in precipitation, clearing the air^{9,10}.

Spectra of water vapour on Mars during 1968-69, taken as Mars was rapidly approaching the Earth⁸, show a shift of the martian H_2O lines relative to the telluric water vapour signature and show much more water on Mars than had recently been found^{11,12}. Humidity can exceed 50% over large areas, which is conducive to occasional precipitation on martian aerosols and changes the atmospheric conditions radically, explaining even abrupt blue haze clearings. Possibly fogs settle on Mars, and rain and snow may originate from reported, heavier, grey and yellow clouds¹⁰.

Consider the orbits of Mars and Earth as being fixed in space (Fig. 1). All months referenced are Earth months. The orbital perihelion of Mars lies in the August sector and the aphelion in the February sector. Mars may, of course, be anywhere in its orbit during August or February, but Earth's near circular orbit provides for closest oppositions around August or September, as for 1971. At close oppositions, Mars is in its northern hemisphere autumn. For oppositions during February and March, Mars is in the middle of the northern hemisphere spring. These are important considerations because most of the data on both blue haze clearings and flares¹³ have been taken during oppositions.

With one polar cap partially or completely dissipated during either martian summer, the humidity will then be highest and conducive to possible precipitation. Blue haze clearings (opposition data) have predominated during martian summers, (both northern and southern) which are favourable seasons for precipitation.

Earth weather probably prejudiced blue haze data, influencing the deficiency during February and March as shown in Fig. 1. Conversely in northern skies it is best to look at Mars when the cool air contributes to greater clarity even at larger distance.

For an April opposition, Mars is not far from its northern summer solstice, favouring increased precipitation in its northern hemisphere, but if this is indeed a factor it implies that opposition blue haze clearings in March and February should be more frequent than are indicated. Another effect, however, is the occurrence of aphelion in the late February sector implying a colder planet overall, with lower humidity due to increased condensation of volatiles. Further correlations of locations of clearings with seasons are indicated.

During our summer months of June, July and August the monthly integrated averages from opposition data¹⁴ of blue haze clearings have been almost a constant. Capen's data¹⁵, however, show a progressive decrease for this period. This total number (approximately 100) has also been low. Most of these data were obtained during oppositions when the warming martian southern hemisphere was emerging from winter, thermal gradients were increasing, with winds carrying dust. The humidity may still be too low for significant precipitation, producing regional atmospheric hazes while northern terrestrial weather hinders observations.

From September to February, the average opposition blue haze clearings per unit time initially increases and is then approximately constant.

The centre of the season of more frequent blue clearings lags the martian southern solstice by about three terrestrial weeks. The duration of this clearing season, however, is almost triple that of the season which centres 3 weeks after the northern summer solstice. This, too, is explicable by increased temperatures over more martian surfaces. This follows from the fact that in the vicinity of northern summer solstice the polar axis inclination favours higher polar surface temperatures, but is thermally opposed by being in the orbital aphelion, whereas in the vicinity of southern summer solstice, polar inclination and orbital eccentricity coordinate to produce higher temperatures, possible precipitation and clearings. Furthermore, while the line of solstices is near to the line of apsides it misses coincidence with it by a few months, causing a long warm period even though the southern summer is shortest. Solar power to Mars is over 40% greater at perihelion than at aphelion because a longer season of more frequent clearings in the austral summer part of the martian orbit is anticipated and supported by the available data (Fig. 1). Apparently orbital eccentricity has greater influence than the inclination of the polar axis.

Recent observations¹⁵ show that, excepting early February, blue clearings have occurred throughout the year. Since Mars presents its smallest image to astronomers during February, atmospheric blurring could easily mask detail. Although a blue deficiency of Mars was observed from Earth during NASA Mariners 6 and 7, none was detected in photographs from the fly-bys with red, blue, and green filters¹⁶. This indicates a terrestrial, faint image, or space contained effect. The high contrast and definition of the Mariner images at close range may have made any obscuration undetectable. Qualitative martian colour contrasts could hardly be established from these unprecedented series of photographs. Opik⁶ feels that Leighton's conclusion of non-existence of the blue haze based on Mariner photographs (which show limb haze) is not justified¹⁰. Blue light photographs taken at Flagstaff, Arizona and Bloemfontein, South Africa, with equivalent refractors, Kodak film and developers consistently show more blue details in the Flagstaff photographs¹⁰. Flashes of light have been reported on Mars^{16,17} which could, it seems, be possible ground water since no volcanic activity is known.

With possible precipitation on Mars, a new perspective is gained in the Mars blue haze clearings and known soil erosion. White and blue clouds, possibly composed of water,

have long been seen which are conducive to rapid atmospheric clearing. Evidence indicates that Earth's summer haze, with appropriate martian conditions could possibly block much blue light from all, or part, of Mars.

There are four factors involved; the inclination of the martian axis of rotation, the eccentricity and orientation of the martian line of apsides, the occurrence of precipitation and dust and terrestrial seeing conditions. These can be combined to explain the distribution of reported opposition blue haze clearings. Photographs or vidicon observations from an orbiting (or lunar based) telescope, or stratoscope, could test our hypothesis by viewing Mars simultaneously from Earth and Earth orbit at times when the surface was obscured in the blue from the ground. Similar tests would be made early in a February when no clearings have been reported. These observations should show how terrestrial weather influences blue haze clearings.

We also speculate that numerous light flashes reported on Mars, coinciding primarily with the respective summer hemispheres at the times of observation, may be reflexions from temporary surface waters formed by precipitation.

E. H. WELLS
D. P. HALE

*Space Sciences Laboratory,
Marshall Space Flight Center,
Alabama 35812*

Received April 7; revised June 10, 1971.

- ¹ Leighton *et al.*, *Science*, **166**, 49 (1969).
- ² Opik, E. I., *Irish Astron. J.*, **9**, 138 (1970).
- ³ *Planets and Satellites* (edit. by Kuiper, G. P., and Middlehurst, B. M.), 383, 434, 554, 555, 557, 562 and 596 (1961).
- ⁴ Hess, S. L., *Meteorology*, **7**, 1 (1950).
- ⁵ Schatzman, E., *CR Acad. Sci.*, **232**, 652 (1951).
- ⁶ Opik, E. I., *J. Geophys. Res.*, **65**, 3057 (1960).
- ⁷ Van der Hulst, *Light Scattering by Small Particles*, 443 (Wiley, New York, 1962).
- ⁸ Schorn, R. A., *et al.*, *Icarus*, **11**, 286 (1969).
- ⁹ Kviv, Z., *Bull. Astron. Inst. Czechoslovakia*, **2**, 1 (1961).
- ¹⁰ Slipher, E. C., *Mars*, 30 (Sky Publishing Company, 1962).
- ¹¹ Spinrad, H., Munch, G., and Kaplan, L. D., *Astrophys. J.*, **137**, 1319 (1963).
- ¹² Kaplan, L. D., Munch, G., and Spinrad, H., *Astrophys. J.*, **139**, 1 (1964).
- ¹³ Newburn, R., *Mars Scientific Model I*, JPL report 606-1, 4.1, 11 (1968).
- ¹⁴ Palm, A., and Basu, B., *Icarus*, **4**, 116 (1965).
- ¹⁵ Capen, C. F., *Icarus*, **12**, 118 (1970).
- ¹⁶ Sabeki, T., *Sky and Telescope*, 144 (February 1955).
- ¹⁷ Pskovskiy, Yuri Pavlovich, Appendix in NASA translation of ref. 8.
- ¹⁸ Katterfel'd, G. N., NASA Technical Translation F-140 from *Priroda*, **8**, 103 (1966).

Permeabilities of Precambrian Onverwacht Cherts and Other Low Permeability Rocks

QUANTITATIVE estimates of rock permeability are of importance in any branch of science or engineering where flow of fluids through rocks is considered. Although there is a large amount of data available for rocks with relatively high permeabilities (exceeding 1 mdarcy), very few data are available for rocks such as shales, cherts, dense carbonates and evaporites which may have permeabilities of less than 1 mdarcy. Permeability measurements on Precambrian cherts help to interpret the results of organic geochemical studies made on these ancient rocks.

The search for geological evidence about the origin and evolution of life on Earth has led to detailed searches for morphological and molecular fossils in Early Precambrian sedimentary rocks, most of which have extremely low permeabilities^{1,2}. The oldest rocks considered so far are the Onver-

wacht Group of the Swaziland Sequence exposed in the Eastern Transvaal of South Africa. The age of this group is at least 3.2×10^9 yr. Fossil-like microstructures as well as polymeric organic matter (commonly called kerogen) have been found in carbonaceous cherts of the Onverwacht Group³, but the biogenic nature of the microstructures is uncertain⁴. The presence of several kinds of molecular fossils in these rocks (*n*-alkanes, isoprenoid hydrocarbons, fatty acids, porphyrins, amino-acids) has also been reported⁵⁻⁷. But the significance of these microstructures and molecular fossils for the origin and evolution of life on Earth is still in question.

Nagy⁸ has recently determined the permeability and porosity of a single sample of Onverwacht chert to be 5.7×10^{-7} mdarcy and 0.5% respectively. He showed that even at this low permeability there is the possibility of contamination of these rocks with organic compounds dissolved in fluids which have flowed through for several thousand million years. This finding has wide implications for the significance of organic compounds found in small concentrations in low permeability rocks of all geological ages.

In January 1968 we began to develop a novel liquid permeameter for measuring very low permeabilities in rocks. Permeabilities and porosities of four samples of chert from four different formations of the Onverwacht Group were measured. For comparison the same measurements were made on a sample of Precambrian Keewatin chert from Canada, a sample of Precambrian Bitter Springs limestone from Australia, and samples of Castile gypsum, Rustler dolomite, and Bone Springs limestone from west Texas.

Permeabilities were determined by a new technique described in detail elsewhere (S. K. Sanyal, R. M. Pirnie, III, G. O. Chen, and S. S. Marsden, jun., to be published). The sample is held in a Hassler sleeve which can be maintained at confining pressures as high as 10,000 pounds inch⁻² with a hydraulic pump. Upstream pressures are maintained by a "thermal pump" which utilizes the thermal expansion of a liquid to create pressures $\leq 1,000$ pounds inch⁻². Pressures are measured with a low displacement, diaphragm-type transducer and permeabilities deduced indirectly from the pressure decline over a period of time.

For the permeability measurements reported here, hydraulic oil was used in both the hydraulic and thermal pumps by contrast with Nagy's choice⁸—a 2,000 p.p.m. NaCl brine. Theoretically permeability of a rock is independent of the flowing fluid but in practice this independence is not strictly valid⁹. Extremely low permeability rocks such as those studied have a higher permeability to air than to water¹⁰. This difference may be due to dehydration of minerals in contact with flowing air (causing an increase in permeability), or the swelling

of clay minerals in contact with flowing water which would cause a decrease in permeability. The second effect can be minimized by use of strong brine or a non-interacting fluid such as hydraulic oil.

Permeability measurements were made at confining pressures between 1,500 and 1,800 pounds inch⁻². An increase in confining pressure on a rock generally reduces its permeability^{9,11,12} and this effect is particularly evident for rocks of very low permeability¹³. Nagy⁸ did not specify the confining pressures used and this to some extent limits a comparison of the results. Permeabilities were measured at various orientations to bedding because in the presence of small scale structures (laminations, banding, fractures, solution channels and so on), rock permeability depends strongly on the directions in which the fluid flows.

Porosity, ϕ , of a sample was calculated from its bulk volume, V_B , its dry weight, W_0 , and its weight after it was saturated with oil under vacuum, W_s , by the relation

$$\phi = \frac{W_s - W_0}{\rho_o V_B}$$

where ρ_o is the density of the oil.

Permeability and porosity values of the samples studied as well as directions of permeability measurements and confining pressures are listed in Table 1. For Onverwacht cherts, permeabilities vary from a value which is too small to be measured by our technique (that is less than 10^{-6} mdarcy) to 2.09×10^{-2} mdarcy, and porosities vary from 0.03 to 0.72%. The chert sample from the Hooggenoeg Formation had a very low permeability which probably approaches the value obtained by Nagy⁸ (5.7×10^{-7} mdarcy). The other Onverwacht chert samples had permeabilities greater by several orders of magnitude. The Keewatin chert from Canada had a permeability of 1.1×10^{-4} mdarcy which is similar to the chert permeability given by Davis¹⁴ (1.9×10^{-4} mdarcy). Permeabilities to air of two Precambrian cherts have been obtained by Smith *et al.*¹⁵; Gunflint chert from Canada had air permeabilities of 1.9×10^{-1} and 2.0×10^{-2} mdarcy and porosities of 0.44 and 0.55%, and Bitter Springs chert had values of 2.2×10^{-1} and 1.7×10^{-1} mdarcy and 0.62 and 1.15%. Thomas *et al.*¹⁶ have shown that air permeabilities are generally slightly higher than liquid permeabilities in tight rocks. Therefore Gunflint and Bitter Springs cherts seem to be about as permeable as the most permeable Onverwacht cherts. Bitter Springs limestone, with a liquid permeability of 4.0×10^{-2} mdarcy and a porosity of 0.24%, is about as permeable and porous as samples of chert from the same formation.

With one exception, too few measurements were obtained to determine whether there was any relationship between perme-

Table 1 Permeability and Porosity Measurements

Sample No.	Name	Age	Porosity (%)	Permeability (mdarcy)	Direction of permeability	Confining pressure (pound inch ⁻²)
Onverwacht cherts						
5-6	Theespruit Formation	Precambrian	0.72	2.09×10^{-2}	Perpendicular to laminations	1,500
5-10	Hooggenoeg Formation	Precambrian	0.03	$< 10^{-6}$	No specific orientation	1,700
5-18	Kromberg Formation	Precambrian	0.12	1.16×10^{-3}	Parallel to laminations	1,600
5-23	Swartkoppie Formation	Precambrian	0.44	1.23×10^{-2}	50° to laminations	1,600
Other rocks						
20	Keewatin chert	Precambrian	0.10	1.1×10^{-4}	No specific orientation	1,600
8-9	Bitter Springs limestone	Precambrian	0.24	4.05×10^{-2}	No specific orientation	1,400
3-2-1 (A)	Castile gypsum	Permian	4.80	1.3×10^{-4}	Perpendicular to laminations	1,600
3-2-2 (A)	Castile gypsum	Permian	3.42	1.6×10^{-4}	Perpendicular to laminations	1,500
3-2-1 (B)	Castile gypsum	Permian	6.43	9.17×10^{-2}	Parallel to laminations	1,800
3-2-2 (B)	Castile gypsum	Permian	4.60	3.10×10^{-2}	Parallel to laminations	1,600
3-1-1	Rustler dolomite	Permian	1.37	4.39×10^{-2}	Parallel to laminations	1,500
3-1-2	Rustler dolomite	Permian	0.41	9.35×10^{-2}	Parallel to laminations	1,500
3-4	Bone Springs limestone	Permian	0.44	4.90×10^{-4}	No specific orientation	1,400

abilities and direction of flow within the rocks. Castile gypsum had permeabilities two to three orders of magnitude higher parallel to the laminations than perpendicular to the laminations. Three out of four samples of Onverwacht chert had measured permeabilities much higher than the single value reported by Nagy⁸. The relationship between measured permeabilities and true permeabilities during geological time is not known but cementation, compaction and metamorphism may have caused significant permeability reduction during the geological past. On the other hand, earth movements may have increased the effective *in situ* permeabilities of these rocks by inducing fracturing which may not be evident in small laboratory samples.

It is quite likely that fluids have been able to flow through these rocks by means of both intergranular and fracture channels. Our data suggest that flow of fluids in these rocks may have been even higher than estimated by Nagy⁸, and consequently many of the Onverwacht cherts may have been host rocks rather than source rocks for some of the organic compounds they now contain.

S. K. SANYAL

Department of Petroleum Engineering,
Stanford University,
Stanford, California 94305

Exobiology Division,
NASA-Ames Research Center,
Moffett Field, California 94035

K. A. KVENVOLDEN

S. S. MARSDEN, JUN.

Department of Petroleum Engineering,
Stanford University,
Stanford, California 94305

Received June 15, 1971.

- ¹ Barghoorn, E. S., and Schopf, J. W., *Science*, **152**, 758 (1966).
- ² Schopf, J. W., and Barghoorn, E. S., *Science*, **156**, 508 (1967).
- ³ Engel, A. E. J., Nagy, B., Nagy, L. A., Engel, C. G., Kremp, G. O. W., and Drew, C. M., *Science*, **161**, 1005 (1968).
- ⁴ Nagy, B., and Nagy, L. A., *Nature*, **223**, 1226 (1969).
- ⁵ MacLeod, W. D., jun., *J. Gas Chromatog.*, **6**, 591 (1968).
- ⁶ Han, J., and Calvin, M., *Nature*, **224**, 576 (1969).
- ⁷ Kvenvolden, K. A., and Hodgson, G. W., *Geochim. Cosmochim. Acta*, **33**, 1195 (1969).
- ⁸ Nagy, B., *Geochim. Cosmochim. Acta*, **34**, 525 (1970).
- ⁹ Afinogenov, Yu. S., *Sniiggims, Novosibirsk*, **6**, 34 (1969).
- ¹⁰ Thomas, L. K., Katz, D. L., and Tek, M. R., *Trans. AIME*, **243**, II-174 (1968).
- ¹¹ McLatchie, A. S., Hemstock, R. A., and Young, J. W., *Trans. AIME*, **213**, 386 (1958).
- ¹² Fatt, I., and Davis, D. H., *Trans. AIME*, **195**, 329 (1952).
- ¹³ Vairogs, J., Hearn, C. L., Dareing, D. W., and Rhoades, V. W., *Proc. 45th Annual Fall Meeting of Soc. Petrol. Engineers of AIME* (1970).
- ¹⁴ Davis, S. N., *Flow Through Porous Media*, 53 (Academic Press, New York, 1969).
- ¹⁵ Smith, J. W., Schopf, J. W., and Kaplan, I. R., *Geochim. Cosmochim. Acta*, **34**, 659 (1970).

Conversion of Oleic Acid to Saturated Fatty Acids in Severn Estuary Sediments

THE upper, biologically active, layers of Recent aquatic sediments contain considerable quantities of saturated and unsaturated long chain fatty acids¹⁻³, presumably from the living and dead organisms found there^{4,5}. Although fatty acids are major constituents of organisms, in the upper layers of sediments, their concentration is of the same order of magnitude as that of sterols^{6,7}, usually minor constituents of organisms. The ratio of fatty acids to total organic carbon in marine sediment is at least ten times lower than in plankton¹, again indicating that fatty acids are subject to rapid changes

in the sediment. Palmitic acid (C_{16:0}) is the most abundant saturated fatty acid in organisms, and oleic acid (C_{18:1}) the most widely distributed and plentiful of all fatty acids^{4,5}. The lower, biologically less active, layers of sediments, contain little or no unsaturated fatty acid; palmitic acid is the most abundant of the fatty acids^{1,2,8}. This difference between the upper and lower layers is unlikely to be the consequence simply of hydrogenation of the carbon-carbon double bonds, for in this case the proportion of stearic acid (C_{18:0}) in the lower layers and the concentrations of saturated fatty acids would be larger. These reactions could occur by chemical and/or biological means, perhaps through the large number of anaerobes present in upper layers of sediments^{9,10}.

Our studies were designed to discover the fate of oleic acid in an aquatic sediment. The basis of our experiments was to add 9,10-³H, 1-¹⁴C-oleic acid to the upper layers of the Recent sediments of the Severn Estuary and then to isolate the labelled products of short term diagenesis. The ratios of ³H to ¹⁴C measured for the various saturated fatty acids obtained are compared with the ³H/¹⁴C ratio for the original oleic acid. We hope to extend the methods described here to other substances of geochemical and environmental chemical interest.

If oleic acid present in sediments is converted into saturated acids (of chain length C₁₂ and above), two biochemical pathways could be operative: (1) the simple hydrogenation of unsaturated carbon-carbon bonds, followed by loss of C₂ units to give the fatty acids of shorter chain length (the degradative pathway); (2) total breakdown of oleic acid by β -oxidation to give C₂ units (probably as acetyl-CoA) followed by a resynthesis through the fatty acid synthetase complex¹¹ (the resynthesis pathway).

If the oleic acid introduced is labelled in the carboxylic carbon with ¹⁴C and in the 9 or 10 positions with ³H (to give 9,10-³H, 1-¹⁴C-oleic acid), the isotopic labels will be incorporated differently into the products of the two pathways outlined here.

In the case of the degradative pathway, which involves the simple loss of C₂ units without total breakdown, the ¹⁴C will be lost from the molecule (for all acids except stearic), whereas all the ³H will be retained. Thus, for fatty acids of length C₁₆ down to C₁₀, the ratio of ³H to ¹⁴C becomes infinitely large, whereas for stearic acid the ratio stays the same as that of the added oleic acid. The ¹⁴C removed from the long chain acids will appear in acetyl-CoA (as 1-¹⁴C-acetyl-CoA), which will have a ratio of ³H to ¹⁴C of 0 to 1.

In the case of the resynthesis pathway, the various enzymatic reactions undergone during β -oxidation and resynthesis from the resulting acetyl-CoA will lead to loss of ³H relative to ¹⁴C. The first turn of the β -oxidation spiral will remove all the ¹⁴C from the molecule as 1-¹⁴C-acetyl-CoA. All the ³H from C-9 will be lost (as NAD³H or FAD³H) as this carbon becomes the terminal carboxylic carbon. The fate of the ³H on C-10 may depend on the stereospecificity of the enzymes involved in the reduction step. Enzymes from various sources¹² have been shown to react stereospecifically with fatty acids and it is likely that the β -oxidation enzymes behave similarly, but their stereospecificity is unknown and either all or none of the ³H could be lost from C-10 of the degraded fatty acid. The acetyl-CoA produced through the β -oxidation spiral could therefore contain anything between zero and 0.5 of the amount of the ³H originally present in the oleic acid (all as 2-³H-acetyl-CoA), and all of the ¹⁴C. This acetyl-CoA is most likely to be incorporated into long chain fatty acids by the fatty acid synthetase complex which is present in many organisms, including *Escherichia coli* and yeast¹¹. All the acetyl-CoA units to be utilized, except one, are first converted to malonyl-CoA before incorporation into the growing fatty acid. The overall effect is to lose approximately 0.67 of the ³H present as 2-³H-acetyl-CoA but to retain all the ¹⁴C. In the case of palmitic acid, the overall result should be a ratio of ³H to ¹⁴C of 8.34 : 1, based on acetyl-CoA having an

Table 1 Radioactivity in Saturated Long Chain Fatty Acids from Sediments Incubated with 9,10-³H, 1-¹⁴C-Oleic Acid (Corrected for Experimental Losses)

Acid	Yields of radioactivity (Ci 10 ⁻⁹)									
	³ H <i>A</i>	¹⁴ C	³ H <i>B</i>	¹⁴ C	³ H <i>C</i>	¹⁴ C	³ H <i>D</i>	¹⁴ C	³ H <i>E</i>	¹⁴ C
Stearic	146.6	5.2	71.2	2.95	12.2	0.41	56.4	2.7	18.9	0.5
Palmitic	167.6	3.8	107.6	4.7	13.7	0.40	70.8	1.07	12.8	0.44
Myristic	80.4	0.7	161.4	3.9	10.3	0.19	46.6	0.27	20.8	0.10
Lauric	74.6	0.2	*	*	4.4	0.03	15.4	0.02	*	*

* Not determined.

100 μ Ci of 9,10-³H-oleic acid and between 2.5 and 10 μ Ci of 1-¹⁴C-oleic acid were injected into the sediment in each experiment. All the results have been normalized to an initial ratio of ³H to ¹⁴C for oleic acid of 20 : 1 (100 μ Ci of ³H). The conditions of extraction and purification of the acids, and the corrections for losses in these procedures, are described in the text. The conditions of incubation were as follows: *A*, laboratory core, incubated for 6 days at 18–20° C; *B*, laboratory core, incubated for 9 days at 18–20° C; *C*, incubated *in situ* for 7 days, August 1970. *D*, incubated *in situ* for 17 days, September–October 1970; *E*, incubated *in situ* for 38 days, September–October 1970. The statistical errors in the ³H values caused by variation in count rates were in all cases less than $\pm 1\%$. The ¹⁴C values will be subject to larger errors because of the lower count rates, and where the quoted yields are less than 10⁻¹⁰ Ci the error may be greater than $\pm 5\%$.

initial ratio of ³H to ¹⁴C of 20 to 1. Experimental verification of this expected ratio comes from the work of Wakil¹³ with the synthetase complex from avian liver, and other studies¹⁴ in rat adipose tissue. Overall, the resynthesis pathway, starting from oleic acid with a ratio of ³H to ¹⁴C of 20 to 1 should generate palmitic acid with a ratio of ³H to ¹⁴C between 0 to 1 and 4.17 to 1, the exact value depending on the ³H loss during β -oxidation. The theoretical ratios for lauric, myristic and palmitic acids formed via the resynthesis pathway are very similar.

Thus the two pathways, degradative and resynthetic, should give end products with very different ratios of ³H to ¹⁴C. Except for stearic acid, the degradative pathway causes a total loss of ¹⁴C, whereas the resynthesis pathway causes an almost total loss of ³H relative to ¹⁴C. We are following only the flow of label and not the total flow of material through each pathway. From the actual ratios of ³H to ¹⁴C observed in the saturated fatty acids extracted from the sediments injected with labelled oleic acid, it is possible to calculate what proportion of the label has come through the degradative pathway compared with that arising by resynthesis.

Incubations of oleic acid in aquatic sediments were carried out either *in situ* in the mud of the Severn Estuary (National Grid Reference ST534832) at a depth of 10 cm below the surface, or in cores (15 $\times$ 3 cm) which had been removed from areas adjacent to the *in situ* sites, and then transferred to the laboratory. The area of study was chosen near the high water mark on a large expanse of mud flats. A thin layer of grey silt covered a 20 cm layer of black thixotropic mud which contained sulphide and elemental sulphur. The sites were covered by the tide for approximately 4 h in each 24 h, and the temperature varied between 5° and 25° C; the laboratory cores were incubated at 18°–20° C. Labelled oleic acid (from the Radiochemical Centre, Amersham, Buckinghamshire) was prepared by mixing aliquots of 1-¹⁴C and 9,10-³H-oleic acid (no additional unlabelled acid was added) to give a ratio of ³H to ¹⁴C of between 10 to 1 and 40 to 1. The resulting oleic acid (up to 100 μ Ci of ³H) was injected (as a 'Tween 80' suspension) so as to cause as little disturbance of the sediment as possible. This represents the addition of a maximum of 0.2 μ mol of free oleic acid.

After incubation for up to 38 days, the cores were removed and extracted for free fatty acids using a modification of the Dole extraction procedure¹⁵, or with a 3 to 1 mixture of toluene/methanol. Carrier fatty acids were added (3 μ mol for each acid) and the extract methylated with BF₃/methanol¹⁶. The methyl esters of the saturated acids were then separated from other material by preparative thin-layer chromatography on silver nitrate-impregnated silica gel¹⁷, and finally separated into individual esters by preparative gas chromatography. The peaks corresponding to the even chain length saturated acids were collected directly in 10 ml. aliquots of toluene scintillator (NE 220: Nuclear Enterprises Ltd, Edinburgh) and the samples assayed for radioactivity using a Nuclear

Chicago mark I liquid scintillation spectrometer. Quench corrections were applied¹⁸.

Control experiments have shown that the isolation procedures used (Dole extraction, methylation, thin-layer chromatography) result in approximately 70% recovery of U-¹⁴C-palmitic acid before preparative gas-liquid chromatographic analysis. Toluene-methanol extraction is less efficient giving 25% recovery of label before gas-liquid chromatography. Similar recoveries have been obtained for 9,10-³H-oleic acid. Table 1 shows the amounts of radioactivity (in nCi) in the saturated fatty acid fraction estimated as being present in sediments after various periods of incubation with 9,10-³H, 1-¹⁴C-oleic acid. The figures listed are estimates calculated using these measured efficiencies of recovery of the saturated fatty acids.

The most striking feature of Table 1 is the low conversion of oleic acid into saturated fatty acids observed for all the incubations. The combined long chain saturated fatty acid fraction extracted from the mud was never found to contain more than 3% of the label from the added oleic acid, even though the labelled oleic acid rapidly disappeared (for example in experiment *B* of Table 1, only 5% remained after 9 days incubation). Thus, most of the label of the starting oleic acid does not appear in the free saturated fatty acid component of the mud. The usual fate of oleic acid added to this particular sediment is probably breakdown to acetyl units, which may then be oxidized to CO₂ and H₂O or incorporated into other compounds such as the carbohydrate constituents of cell walls.

Table 2 shows the ratios obtained after various incubation times, with 9,10-³H, 1-¹⁴C-oleic acid. There is a definite trend in the ³H to ¹⁴C ratio, which increases with decreasing chain length. We have already stated that acids produced by the degradative pathway would have a ratio of ³H to ¹⁴C of infinity, whereas those formed by the resynthesis pathway would have ratios of ³H to ¹⁴C between 0 to 1 and 4.2 to 1, starting with that for oleic acid—20 to 1. A combination of the pathways would give intermediate ratios. A qualitative

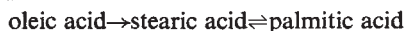
Table 2 Ratios of ³H to ¹⁴C measured in Saturated Fatty Acids from Severn Estuary Sediments after Injection of 9,10-³H, 1-¹⁴C-Oleic Acid

Acid	<i>A</i>	<i>B</i>	<i>C</i>	<i>D</i>	<i>E</i>
Stearic	29 : 1	24 : 1	30 : 1	21 : 1	38 : 1
Palmitic	44 : 1	23 : 1	34 : 1	66 : 1	29 : 1
Myristic	112 : 1	41 : 1	54 : 1	170 : 1	215 : 1 *
Lauric	440 : 1 *	—	142 : 1 *	627 : 1 *	

Procedures for incubation and isolation of the acids are given in the text. The ratios of ³H to ¹⁴C used in the injected oleic acid varied between 10 to 1 and 40 to 1; all the results have been normalized to a starting ratio for oleic acid of 20 to 1. The conditions of incubation (*A*–*E*) are given beneath Table 1.

* These values are subject to errors of greater than $\pm 5\%$.

picture can be readily inferred from the data in Table 2. Lauric acid and myristic acid, which have high ratios of ^3H to ^{14}C , seem to arise chiefly through the degradative pathway, while palmitic acid, which has a ratio nearer that of oleic acid, seems to be formed through both pathways. The position with stearic is more complex, as a ratio of ^3H to ^{14}C greater than that of oleic acid is impossible according to the scheme outlined above. Some of the stearic acid, however, may be formed through a recycling pathway involving palmitic acid:



A ratio of ^3H to ^{14}C greater than that of oleic acid would then be obtained. Such recycling of metabolites is well known in biochemistry^{13,19}, and it is quite likely that recycling is also occurring here.

Table 3 Calculated Fractions of Labelled Saturated Fatty Acids derived by the Degradative Pathway

Acid	A	B	C	D	E
Palmitic	0.67 (0.69)	0.48 (0.53)	0.60 (0.63)	0.76 (0.77)	0.56 (0.60)
Myristic	0.84 (0.85)	0.65 (0.66)	0.72 (0.73)	0.90	0.92
Lauric	0.96	—	0.88	0.97	

The observed ratios of ^3H to ^{14}C used to calculate these results are taken from Table 2, and the conditions of incubation (A–E) are given in the footnote to Table 1. The values in this table have been calculated using the formula

$$x = \frac{c_{\text{res}}t_{\text{obs}} - c_{\text{obs}}t_{\text{res}}}{c_{\text{obs}}(t_{\text{deg}} - t_{\text{res}}) + t_{\text{obs}}(c_{\text{res}} - c_{\text{deg}})}$$

where x is the fraction of acid formed through the degradative pathway. t_{res} to c_{res} is the theoretical ratio of ^3H to ^{14}C obtained for an acid formed through the resynthesis pathway. t_{deg} to c_{deg} is the theoretical ratio ^3H to ^{14}C through the degradative pathway. t_{obs} to c_{obs} is the observed ratio of ^3H to ^{14}C in the purified methyl esters of the long chain fatty acids. The first figure of each value is that assuming minimal loss of ^3H through the resynthesis pathway; that in parentheses is assuming total loss of ^3H . Where only one figure is given, the two results differ by less than 0.01. Stearic acid has been omitted from this table because recycling between stearic and palmitic acids cannot be estimated.

The results in Table 3 provide an approximate order for the proportion of individual labelled saturated fatty acids formed from labelled oleic acid by the degradative pathway:



A significant portion of the labelled palmitic acid is formed on the resynthesis pathway, which is probably a reflexion of the fact that the fatty acid synthetases of many organisms produce predominantly palmitic acid¹². Thus shorter chain acids (lauric and myristic acids) formed through the resynthesis pathway would have been produced by partial degradation of palmitic acid, with concomitant dilution of label by the cellular pool of palmitic acid.

Several assumptions have been made in this treatment of the results, and these will be discussed briefly. It has been assumed that the major pathways for the transition of label from oleic acid to the saturated acids involve either removal of a small number of C_2 units by means of β -oxidation (degradative pathway), or total breakdown to acetyl-CoA followed by resynthesis (resynthesis pathway). These are the principal pathways of fatty acid metabolism in most organisms²⁰, and we have assumed that other pathways, such as α -oxidation^{21,22}, are of little importance. Our calculations of the proportions of flow of label through the two pathways also require the assumption that any acid which has been formed by the resynthesis pathway is not then subsequently totally broken down again. If this occurs, our estimates of the flow through the resynthesis pathway will be too low, for the label will be lost from the system. During the whole incubation period the ratio of ^3H to ^{14}C for oleic acid should remain constant, and any variation will cause

errors in the calculations of the fractions of labelled acids derived by the two pathways. The oleic acid fractions recovered after the incubations had ratios of ^3H to ^{14}C within 10% of the starting ratios.

The composition and abundance of biolipids and geolipids present in Recent sediments vary markedly with depth. Measurement of these differences provides one approach to the problem of studying the path of carbon during diagenesis. Farrington and Quinn²³ measured fatty acids at various depths in Recent sediment from Narragansett Bay, Rhode Island. They concluded that $\text{C}_{16:0}$ is the predominant acid at the depths studied (surface to 52 cm) and that the concentrations of the unsaturated fatty acids $\text{C}_{16:1}$ and $\text{C}_{18:1}$ decrease more rapidly with depth than do the saturated fatty acids. Brogden²⁴ has studied the anaerobic decomposition of zooplankton in a mixture of sediment and seawater which had been sterilized and inoculated with bacteria from marine sediments. The quantities of $\text{C}_{16:1}$ and $\text{C}_{18:1}$ acids fell rapidly over the period of the experiment, whereas the concentration of saturated fatty acids increased. Brogden suggested hydrogenation of the unsaturated fatty acids.

The novel approach described here involves direct introduction of radiolabelled biolipids into a Recent sediment. It provides specific and detailed information on the fate of a labelled compound introduced into the sediment. The method involves only minor disturbance of the ecosystem and allows estimates to be made of the fluxes through various biochemical pathways. This work substantiates the current view^{1,23} that oleic acid rapidly disappears after burial in a Recent aquatic sediment. We have shown that some of the atoms of oleic acid reappear in the saturated normal fatty acid fraction, although the conversion is small. It is probable that most of the oleic acid is metabolized by degradation of the carbon skeleton through β -oxidation to acetyl-CoA, followed by conversion to CO_2 and H_2O or incorporation into non-lipid cellular components. We have calculated the proportions of the various saturated acids which have been produced from labelled oleic acid by the pathways we have considered, namely the degradative and resynthesis pathways (Table 3). The majority of the label was incorporated through the degradative pathway with retention of part or all of the original carbon skeleton of the oleic acid. The proportion is highest for lauric and myristic acids. But there are difficulties in deriving detailed inferences from information of this sort. The two pathways must differ markedly in their capability for dilution of label. Thus, the degradative pathway is unlikely to be subject to a large dilution of label and the radioactivity incorporated can be used to estimate the rate of carbon flow in this pathway. In contrast, in the resynthesis pathway the label passes through the acetyl-CoA pool of the cell. Acetyl-CoA is a key metabolite in most cells, and several pathways lead into and away from this pool. Calculations using the flow of label through the resynthesis pathway probably underestimate the total carbon flow from acetyl-CoA to long-chain fatty acids.

Our experiments certainly show that oleic acid present in a Recent sediment can be converted into saturated fatty acids with retention of carbon skeleton over a period of a few days. The process is almost certainly effected microbiologically. Whether this represents a process which contributes significantly to the content of the saturated fatty acid fraction found in Ancient sediments is uncertain. The rapid loss of oleic acid during the short periods of our experiments, coupled with the slight incorporation into saturated fatty acids through both pathways, suggests, however, that the saturated fatty acids found in compacted Recent and Ancient sediments are not derived directly from the fatty acids originally contributed to surface layers, but are derived from a number of precursors through many metabolic pathways.

On occasions the steady process of sedimentation may be interrupted by the sudden deposition of large quantities of sediment as a result of major floods or earthquakes, and in

this case the sediment might be buried complete with a greater abundance of biolipids and their immediate products of alteration. Another mechanism whereby fatty acids might escape direct biological alteration could be encapsulation within a seed or nut²⁵. Our prime concern in this study has been to investigate the processes of hydrogenation which are believed to occur early in the formation of a sediment. In deeper layers, as lithification proceeds, free water is gradually removed and bacterial activity ceases. As the temperature and pressure rise, slow maturation processes begin. Hydrogen transfer is a part of these slow maturation processes. Thus radiolabelled cholesterol, when intimately mixed with a sample of pulverized Green River shale (Eocene) and heated in a sealed tube at 200° for 1,000 h, afforded various radiolabelled products which included 5 α and 5 β -cholestane (unpublished results). The relationships between biolipids incorporated into a freshly forming sediment and those geolipids remaining after the initial period of biological and chemical diagenesis probably now encompass the following: (1) complete retention of structure as in the odd-numbered alkanes originating in the surface waxes of plant debris; (2) partial retention of structure, as in the saturated fatty acids formed by the degradative pathway in the present study, and partial or complete retention of stereochemistry as in isoprenoid acids derived, presumably, from phytol²⁶; (3) complete breakdown and resynthesis as in the saturated fatty acids formed by the resynthesis pathway.

This study indicates that hydrogenation processes play a part in the early diagenesis of fatty acids. We are investigating the fate of several unsaturated biolipids for which saturated geolipid analogues are encountered in Ancient sediments. It seems likely that saturated geolipids may have a dual origin—biologically effected hydrogenation during early diagenesis, and thermally and catalytically induced hydrogen transfer processes during maturation. These hydrogenation processes are the only ones tenable unless biological sources can be found for fully saturated geolipids such as steranes, triterpanes and tetraterpanes and related compounds^{27,28}.

We thank Drs G. Ware, R. Denton, V. Collins, L. Morris and B. Nicholls and Mr F. J. H. Mackereth for advice, equipment and facilities. We thank Mrs Pat Muir for collecting samples and for the microbiological studies and Mr D. Morgan and Miss J. Hay for technical assistance. We thank the SRC, NERC and NASA for financial support. P. J. E. is a Beit Memorial research fellow.

M. M. RHEAD
G. EGLINTON
G. H. DRAFFAN*

Organic Geochemistry Unit,
School of Chemistry, and

P. J. ENGLAND

Biochemistry Department,
University of Bristol,
Bristol 8

Received March 15; revised June 4, 1971.

* Present address: Clinical Pharmacology Unit, Department of Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London W12.

- ¹ Parker, P. L., and Leo, R. F., *Science*, **148**, 373 (1965).
- ² Peterson, D. H., thesis, Univ. Washington (1967).
- ³ Parker, P. L., in *Organic Geochemistry* (edit. by Eglinton, G., and Murphy, M. T. J.) (Springer, Berlin and Heidelberg, 1969).
- ⁴ Shorland, F. B., in *Chemical Plant Taxonomy* (edit. by Swain, T.) (Academic Press, London and New York, 1963).
- ⁵ Gunstone, F. D., *An Introduction to the Chemistry and Biochemistry of Fatty Acids and their Glycerides* (Chapman and Hall, London, 1967).
- ⁶ Parker, P. L., *Contributions in Marine Science*, **4** (1967).
- ⁷ Schwendinger, R. B., and Erdman, J. G., *Science*, **144**, 1575 (1964).
- ⁸ Rosenfeld, W. D., *Arch. Biochem. Biophys.*, **16**, 263 (1948).
- ⁹ Oppenheimer, C. H., *Geochim. Cosmochim. Acta*, **19**, 244 (1960).

- ¹⁰ Zobell, C. E., and Upham, H. C., *Bull. Scripps Inst. Oceanog.*, **5**, 239 (1944).
- ¹¹ Lynen, F., *Biochem. J.*, **102**, 381 (1967).
- ¹² Morris, L. J., *Biochem. J.*, **118**, 681 (1970).
- ¹³ Wakil, S. J., *J. Lipid Res.*, **2**, 1 (1960).
- ¹⁴ Rognstad, R., and Katz, J., *Arch. Biochem. Biophys.*, **127**, 437 (1968).
- ¹⁵ Dole, V. P., and Meinertz, H., *J. Biol. Chem.*, **235**, 2595 (1960).
- ¹⁶ Metcalfe, L. D., Schmitz, A. A., and Belka, J. R., *Anal. Chem.*, **38**, 514 (1966).
- ¹⁷ Morris, L. J., *Chem. Ind.*, 1238 (1962).
- ¹⁸ Williams, M. A., Cope, G. H., Jackson, J. L., and Hill, P., *Biochem. J.*, **118**, 379 (1970).
- ¹⁹ Denton, R. M., and Randle, P. J., *Biochem. J.*, **104**, 423 (1967).
- ²⁰ Mahler, H. R., and Cordes, E. H., *Biological Chemistry* (Harper and Row, New York, 1966).
- ²¹ Mead, J. F., and Levis, G. M., *J. Biol. Chem.*, **238**, 1634 (1963).
- ²² Hajra, A. K., and Radin, N. S., *J. Lipid Res.*, **4**, 270 (1963).
- ²³ Farrington, J. W., and Quinn, J. G., *Nature*, **230**, 67 (1971).
- ²⁴ Brogden, W. B., thesis, Univ. Florida (1968).
- ²⁵ Godwin, H., *The History of the British Flora* (Cambridge University Press, Cambridge, 1956).
- ²⁶ Cox, R. E., Maxwell, J. R., Eglinton, G., Pillinger, C. T., Ackman, R. G., and Hooper, S. N., *Chem. Commun.*, 1639 (1970).
- ²⁷ Henderson, W., Wollrab, V., and Eglinton, G., in *Advances in Organic Geochemistry* (edit. by Schenck, P. A., and Havenear, I.) (Pergamon, London, 1968).
- ²⁸ Streibl, M., and Herout, V., in *Organic Geochemistry* (edit. by Eglinton, G., and Murphy, M. T. J.) (Springer, Berlin and Heidelberg, 1969).

Late Quaternary Stratigraphy and Radiocarbon Chronology of Water Level Fluctuations in Lake Keilambete, Victoria

WHEN compared with the northern hemisphere, the southern hemisphere has provided little palaeoclimatic information on the past 30,000 yr. A systematic study of lakes is under way to help establish a reliable palaeoclimatic sequence covering this period in south-east Australia and to explore possible correlations with worldwide climatically controlled events. This is a preliminary report of the stratigraphic and radiocarbon analysis of deposits from Lake Keilambete, located within the western Victorian volcanic province near Kerang 190 km west-south-west of Melbourne (Fig. 1).

The lake occupies the crater of a maar—a volcanic eruption crater lying below the general level of the surrounding country

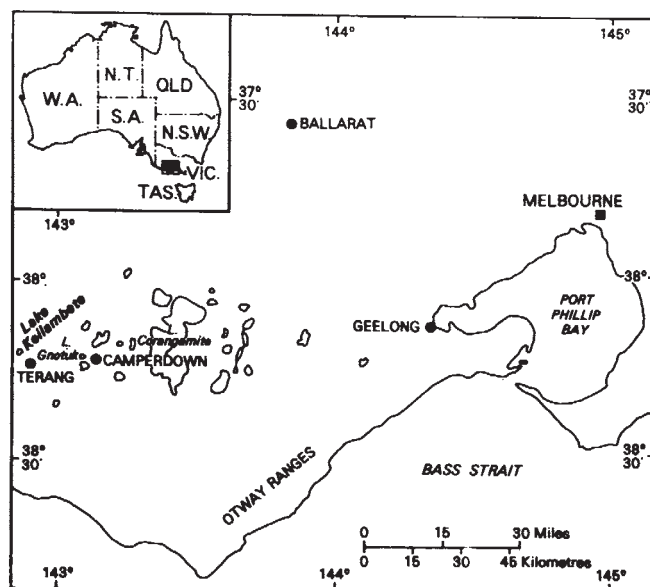


Fig. 1 Diagram showing location of Lake Keilambete.

and surrounded by a low tuff ring. The crater forms an internal drainage basin 1.8 km in diameter; it has a catchment area of 4.2 km² and is controlled by a relatively simple hydrologic system. Lake level responds sensitively to changes in precipitation or evaporation and lake sediments offer a means of reconstructing the sequence of climatic oscillations in late Quaternary time.

The lake derives water from three sources: direct precipitation, runoff from the crater slopes, and a small (almost negligible) ground water increment. Water is lost by evaporation alone as a thick clay effectively prevents water loss through the lake floor. During former high water phases, overflow may have occurred through the lowest point of the crater rim.

Throughout the period of European settlement, the crater has remained filled with water but has not overflowed. Compared with the low salinities of the contributing waters, the high salinity of the lake (60,000 p.p.m.) indicates that it has remained a closed basin for a long time. Lake stratigraphic data are available from two sources. First, around the margins of the lake, partly lithified lake deposits unconformably overlie Miocene calcarenite, the local bedrock of the region. Near the water's edge approximately 6 m of coarse, bedded and rounded detritus alternates with fine white marls. The coarse detritus is reworked volcanic fragments and contains lacustrine mollusca (*Coxiella* and *Limnea*). It is interpreted as a shallow lake deposit. The marls resemble carbonates forming in the lake today. Second, a series of ten cores taken from the shoreline out towards the deepest part of the lake provides a record of the recent lake deposits.

Table 1 Radiocarbon Dates from Lake Keilambete*

Lab. No.	Description	¹⁴ C age (yr BP †)
N-517	11 to 21 cm, core 4	610 ± 110
N-518	21 to 33 cm, core 4	935 ± 110
N-390	Tree submerged by lake waters and overlain by lacustrine sediments	1,890 ± 115
N-519	55 to 65 cm, core 4	1,970 ± 110
N-520-1	79 to 90 cm, core 4	2,410 ± 120
N-521-1	102 to 112 cm, core 4	2,600 ± 110
N-522-1	130 to 140 cm, core 4	2,970 ± 120
N-523-1	165 to 175 cm, core 4	3,580 ± 125
N-524-1	190 to 200 cm, core 4	4,200 ± 125
N-525-1	235 to 245 cm, core 4	5,250 ± 135
N-526-1	290 to 300 cm, core 4	6,440 ± 145
N-527-1	325 to 345 cm, core 4	7,850 ± 165
N-528	395 to 412 cm, core 4	14,300 ± 300
ANU-199	Carbonate from upper marl below disconformity	20,340 ± 500
N-567	Carbonate from upper marl band interbedded in reworked volcanic agglomerate	21,600 ± 650
N-566	Carbonate from marl near base of lake sequence	29,100 ± 1,250

* Data from refs. 1 and 2.

† Except where specified, all dates were obtained from total organic carbon.

The stratigraphic section (Fig. 2), generalized from both outcrop and core sequences, reveals a basal unit of volcanic agglomerate and marl representing earliest lake deposits on which a soil horizon has formed. This is disconformably overlain by a 4 m sequence of unconsolidated, dark, organic-rich muds which alternate with pale carbonate bands. This upper unit was deposited since the last major drying, represented by the soil horizon below 400 cm.

Within the upper unit several sandy horizons in the otherwise fine textured deposits denote important facies variations related to changes in water level. In present day lake sediments, sands similar to those at 100, 125 and 400 cm (Fig. 2) are restricted to water depths of less than 3 m while clays are characteristic of deeper water. Assuming that the depth control of texture found in the relatively simple hydrodynamic system

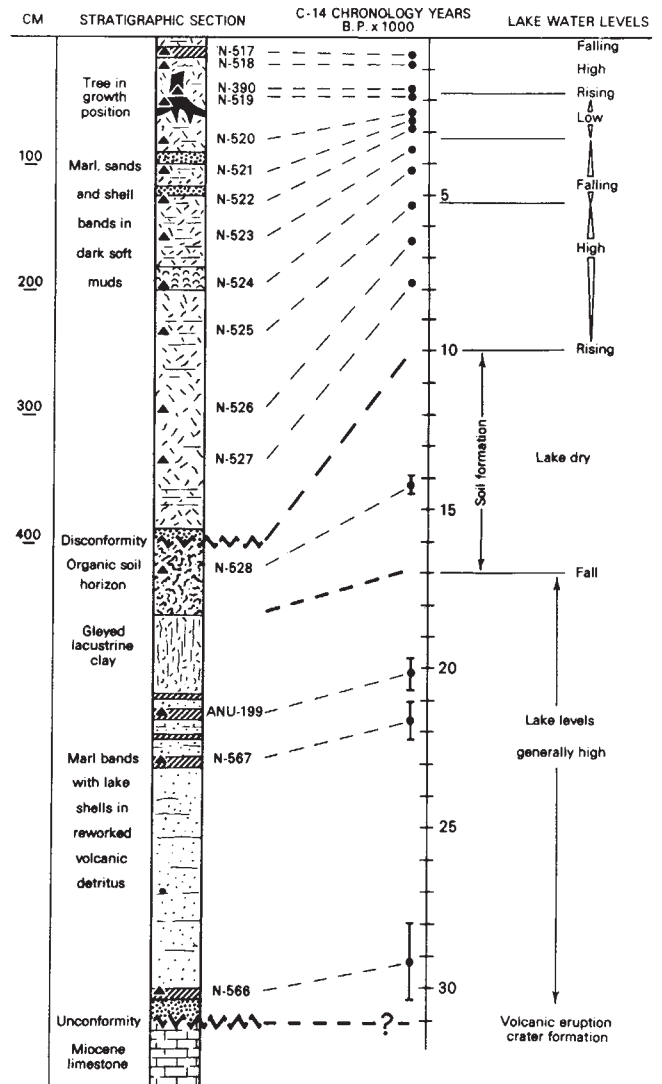


Fig. 2 Generalized stratigraphic section through sediments of Lake Keilambete showing ¹⁴C chronology and sequence of reconstructed water level oscillations. Standard age deviations are shown only when values exceed ±250 radiocarbon years.

of the lake today also applied in the past, alternations of coarse and fine sediments in the cores can be directly related to past water level oscillations. Moreover, the occurrence of organic matter throughout the upper unit permits the facies variations to be accurately dated, thereby establishing the age of the oscillations and of the hydrologic changes which controlled them.

Radiocarbon dates representing 10 cm vertical thicknesses were obtained from core samples throughout the upper lake muds, from organic material in the buried soil horizon and from lake marls in the lower lithified unit. Additional ¹⁴C analyses of carbonates from the upper lake muds¹ will be discussed elsewhere. The radiocarbon dates (Table 1) form a consistent series. Moreover, the ¹⁴C ages of organic remains in the upper muds are consistent with ages based on estimated rates of deposition. They show no measurable initial error due to incorporation of old carbon into the organic content. The basal lacustrine sediments indicate that lake-full conditions existed from about 30,000 BP until after 18,000 BP. Some time between 15,000 and 18,000 BP the lake dried and remained in this state for several thousand years, long enough to permit soil development on older lake sediments. Ephemeral swampy conditions probably existed at this time.

The age of the next water level rise, estimated by extrapolating the ¹⁴C ages to the base of the cores (assuming approximately constant rates of deposition), began about 10,000 BP.

The lake then remained deep for several thousand years and may even have overflowed. About 5,500 BP a change in the hydrologic environment is reflected in an increase in carbonate production and the eventual coarsening of bottom sediments, culminating in the deposition of shallow facies at 3,100 BP. At 3,100 BP water level rose slightly and then fell again, maintaining this low position until about 2,000 BP. Trees found in positions of growth near and below present water level and dated at 1,890 BP (N-390) were submerged by rising waters at this time. The trees belong to the last period when water level was below that of today. The lake rose and remained deep until after European settlement. In the late nineteenth century it began to fall and in 1969 stood at its lowest level for the past 1,900 yr.

In nearby Lake Gnotuk, submerged trees have been dated at $1,865 \pm 85$ BP (GX-0149, E. D. Gill, personal communication). Synchronous oscillations in lakes 20 km apart provide strong evidence that the controlling factor was climate rather than local hydrologic variations. Furthermore, the major drying between 18,000 and 15,000 BP is in close agreement with evidence documented from lakes in different hydrologic settings in north Victoria and south-west New South Wales recorded by J. M. B.³ The rise at about 10,000 BP, reflecting an increase in precipitation or decrease in temperature, cannot yet be correlated accurately with events elsewhere in Australia, although Kershaw⁴ suggests a wet period in north Queensland at this time.

Effects of a Post-Glacial thermal maximum are weakly represented by a slow fall in water level and a corresponding rise in carbonate production from 5,500 to 3,100 BP. The magnitude and implied rates of these changes suggest conditions little different from those in the area today. The data therefore do not support the hypothesis of widespread Post-Glacial aridity so widely claimed in Australia⁵⁻⁷. Although temperatures from 5,500 to 3,100 BP may have been slightly higher than in the preceding interval, the degree of aridity would almost certainly not have been sufficient to cause the vegetation destruction and widespread landscape instability which are sometimes attributed to events of this time.

Conditions moderated at 3,100 BP when a slight rise of lake level occurred, but the major rise marking the beginning of the next relatively wet period did not occur until 1,900 BP.

Oscillations in Lake Keilambete can be matched in other lakes in a variety of settings in south-east Australia supporting the interpretations of the water level changes discussed here. The sequence of palaeoclimatic conditions probably has general application through south-east Australia and it may help provide a reliable basis for climatic correlations within Australia and between different continents in both hemispheres.

We thank Mr J. N. Jennings and Mr C. D. Ollier who read and criticized the manuscript, and Mr Keith Mitchell for drawing the figures.

J. M. BOWLER

Department of Biogeography and Geomorphology,
Australian National University,
Canberra, ACT

TATSUJI HAMADA

Institute of Physical and Chemical Research,
Yamato-Machi Saitama Prefecture,
Japan

Received June 14, 1971.

¹ Yamasaki, F., Hamada, T., and Hamada, C., *Radiocarbon*, **12**, 559 (1970).

² Polach, H. A., Lovering, J. F., and Bowler, J. M., *Radiocarbon*, **12**, 1 (1970).

³ Bowler, J. M., in *Aboriginal Man and Environment in Australia* (edit. by Mulvaney, D. J., and Golson, J.), 47 (Australian National University, 1971).

⁴ Kershaw, A. P., *New Phytol.*, **69**, 785 (1970).

⁵ Brown, W. R., *Proc. Linn. Soc. New South Wales*, **70**, 5 (1945).

⁶ Downes, R. G., *Austral. J. Agric. Res.*, **5**, 448 (1954).

⁷ Gill, E. D., *Austral. J. Sci.*, **17**, 204 (1955).

Asbestos Fibres in Beverages and Drinking Water

THE hazards of inhaling asbestos fibres¹⁻⁴, the high incidence of gastrointestinal cancer^{1,2,5-7} and abdominal neoplasms^{3,8,9} among asbestos workers and the fact that asbestos fibres may penetrate the mucosa of the stomach and the intestine of animals^{10,11} are known and so it is important to study the distribution of asbestos fibres in the environment. Asbestos fibres have already been found in beer¹², and the occurrence of fibres 0.5 nm in length and 0.01 nm in diameter suggests that such material may find its way through filtering systems into city drinking water supplies, and this we have been able to confirm.

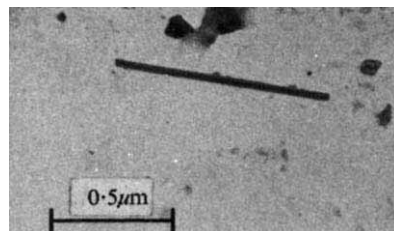


Fig. 1 Electron micrograph of asbestos fibre isolated from beer (enlarged 31,000 times).

The asbestos fibres in 400 ml. samples of beverages and water were concentrated by centrifuging in excess of 16,000g, drying at 100° C and ashing at 500° C¹². The ash was taken up in 1 ml. of distilled water and agitated in an ultrasonic vibrator to disperse the fibres and ash particles¹². Five μl. of this dispersion was evaporated with the aid of a heat lamp on 200 or 400 mesh carbon coated copper grids. Using a Siemens Elmiscop IA electron microscope at 80 kV and a magnification of 20,000, the number of fibres in each of five or ten grid holes was counted, and corrected for dilution. Precautions were taken to prevent contamination of samples from extraneous airborne fibres and no fibres were detected in distilled water

Table 1 Asbestos Fibres in Beverages and Water

Sample	Source*	No. of fibres × 10 ⁶ l. ⁻¹
Beer	Canadian 1	4.3
Beer	Canadian 2	6.6
Beer	USA 1	2.0
Beer	USA 2	1.1
Sherry	Canadian	4.1
Sherry	Spanish	2.0
Sherry	South African	2.6
Port	Canadian	2.1
Vermouth	French	1.8
Vermouth	Italian	11.7
Soft drink	Ginger ale	12.2
Soft drink	Tonic water I	1.7
Soft drink	Tonic water II	1.7
Soft drink	Orange	2.5
Tap water	Ottawa, Ottawa river (F)	2.0
Tap water	Toronto, Lake Ontario (F)	4.4
Tap water	Montreal, St Lawrence river (F)	2.4
Tap water	Hull, Quebec, Ottawa river (NF)	9.5
Tap water	Beauport, Quebec, St Lawrence river (6 km below Quebec City) (NF)	8.1
Tap water	Drummondville, Eastern Townships, Quebec, St Francois river (F)	2.9
Tap water	Asbestos, Eastern Townships, Quebec, Nicolet river (F)	5.9
Tap water	Thetford Mines, Eastern Townships, Quebec, Lac à la Truite (NF)	172.7
Melted snow	Ottawa, top 30 cm (2-3 weeks precipitation)	33.5
River water	Ottawa river, at Ottawa	9.5

* F, Filtration plant used; NF, no filtration plant used.

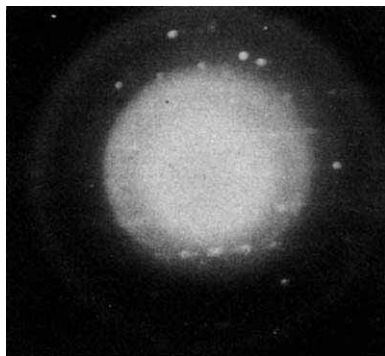


Fig. 2 X-ray diffraction pattern of asbestos fibre shown in Fig. 1.

blanks which were treated in the same manner as the liquid samples.

Twelve brands of Canadian beer, wine, sherry and port, six of American beer, six of European wine, vermouth and sherry and two of South African sherry were examined and all were found to contain asbestos fibres. Counts made on some of these brands are reported in Table 1. The fibres in Canadian beer (Fig. 1), sherry and water were identified as being chrysotile asbestos by comparing their electron diffraction pattern (Fig. 2) with that of pure chrysotile asbestos. Some of the European samples contained fibres of amphibole asbestos. Many asbestos fibres were hidden in clumps of debris such as mineral matter in water and diatomaceous earth which is used as a filtering aid in beer. As a result, the number of fibres reported in Table 1 must be considered a minimum estimate of those actually present. Most of the fibres found in Canadian beer and in filtered city water were less than 1 μm in length while most samples of wine, some American beer and unfiltered melted snow or river water also contained a considerable number of asbestos fibres ranging from 1 to 15 μm in length.

Tap water from three major Canadian cities, Ottawa, Toronto and Montreal, combined from 2.0 to 4.4 million fibres per litre. All of these cities have filtration systems and Ottawa tap water, drawn from the Ottawa river, had considerably fewer fibres than unfiltered river water. Many of Canada's major asbestos mines are located in the Eastern Townships of Quebec and filtered tap water from towns in this area did not differ greatly from that in the larger cities but unfiltered tap water in Thetford Mines, drawn from a small lake in the heart of an asbestos mining area, was exceptionally high in fibre content. The high level of asbestos fibres in melted snow in the Ottawa area gives some indication of one source of asbestos in surface waters.

The question as to the hazard of orally ingested asbestos has not yet been resolved. The amounts by weight of the asbestos found in these samples were exceedingly small—about 1 μg per 10 million fibres—obtained from counts on 1 mg samples of fibres of approximately the same size as those found in the beverages and water. Further work is required to determine the extent to which the fibres penetrate the walls of the digestive tract, the degree to which they may be transported to other organs of the body and the biological effects of their residence in various tissues. Studies of this nature are now in progress.

We thank Mr D. Campbell and Mr G. Bergeron for technical assistance and the Food and Drug Directorate, Department of National Health and Welfare, Ottawa, for obtaining the water samples.

H. M. CUNNINGHAM
R. PONTEFRAC

Food and Drug Directorate,
Department of Health and Welfare,
Ottawa

Received May 3; revised June 14, 1971.

- ¹ Mancuso, T. F., and Coulter, E. J., *Arch. Environ. Health*, **6**, 210 (1963).
- ² Selikoff, I. J., Churg, J., and Hammond, E. C., *J. Amer. Med. Assoc.*, **188**, 142 (1964).
- ³ Hourihane, D. O., *Ann. NY Acad. Sci.*, **132**, 647 (1965).
- ⁴ Wagner, J. C., *Ann. NY Acad. Sci.*, **132**, 575 (1965).
- ⁵ Enterline, P. E., and Kendrick, M. A., *Arch. Environ. Health*, **15**, 181 (1967).
- ⁶ Knox, J. F., Holmes, S., Doll, R., and Hill, I. D., *Brit. J. Indust. Med.*, **25**, 293 (1968).
- ⁷ Newhouse, M. L., and Wagner, J. C., *Brit. J. Indust. Med.*, **26**, 302 (1969).
- ⁸ Keal, E. E., *Lancet*, ii, 1211 (1960).
- ⁹ Hinson, K. F. W., *Brit. J. Dis. Chest*, **59**, 121 (1965).
- ¹⁰ Smith, W. E., Miller, L., Elsasser, R. E., and Hubert, D. D., *Ann. NY Acad. Sci.*, **132**, 456 (1965).
- ¹¹ Westlake, G. E., Spjut, H. J., and Smith, M. N., *Lab. Invest.*, **14**, 2029 (1965).
- ¹² Biles, B., and Emerson, T. R., *Nature*, **219**, 93 (1968).

BIOLOGICAL SCIENCES

Molecular Conformation of Deoxyuridine

THE structure determination of deoxyuridine was undertaken as part of a series of conformational studies of nucleic acid components. Such studies give information about the preferred conformation of nucleosides and nucleotides^{1,2}, and may clarify the conformational differences and changes that occur in nucleic acids. The conformational parameters of interest in deoxyuridine are the pucker of the sugar ring, the torsion angle of the sugar relative to the base about the glycosidic bond and the orientation of the C(5')-O(5') bond about the C(4')-C(5') bond.

In ribose and deoxyribose nucleosides, the most common type of pucker of the sugar is either C(2') endo or C(3') endo. In the deoxynucleosides the C(2') endo pucker is by far the most common. Since one of the nucleosides with a C(3') exo pucker is thymidine³ it was of particular interest to know whether deoxyuridine would also have this type of pucker. Even the same nucleoside in different environments can have a different type of pucker⁴, but these differences have only been observed in ribose nucleosides. There is therefore a need for further studies of deoxy nucleosides and nucleotides.

Crystals of deoxyuridine were obtained from aqueous solutions. The unit cell is monoclinic with dimensions $a=7.91$ Å, $b=6.71$ Å, $c=18.77$ Å, $\beta=96.6^\circ$ and the space group is $P2_1$. There are four molecules in the unit cell and therefore two molecules in the asymmetric unit. Intensity data were collected with a Hilger and Watts linear diffractometer using molybdenum $K\alpha$ radiation. The structure was solved by Patterson interpretation methods. The first step was the determination of the orientation of the plane of the pyrimidine bases in the unit cell using the $I(\theta, \phi)$ function of Tollin and Cochran⁵. The orientation of the pyrimidine base within the plane was determined by means of a rotation function⁶, and the positions of the molecules relative to the 2_1 axis were found using the $Q(X_0Z_0)$ function of Tollin⁷. Full details of the structure determination will be given elsewhere. The R factor at the present stage of refinement is 0.08.

The conformation of both molecules in the asymmetric unit is very similar. The pucker of both sugars is C(2') endo, or C(2') endo-C(3') exo with respect to the three-atom plane C(1')-O(1')-C(4'). Displacements of the C(2') atoms in molecules I and II relative to this plane are 0.508 and 0.383 Å respectively, and of atoms C(3') they are 0.111 and 0.210 Å respectively. Thus the sugar pucker in deoxyuridine is like that in the majority of deoxynucleosides and differs from that in thymidine.

The glycosidic torsion angle, ϕ_{CN} , defined by Donohue and Trueblood⁸, is -28° in molecule I and -24° in molecule II.

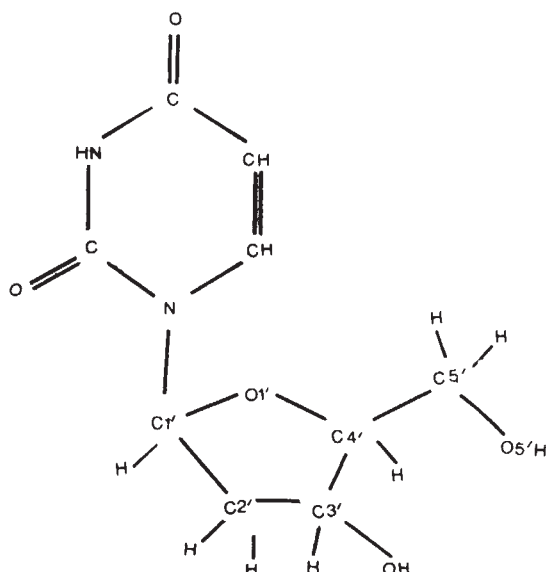


Fig. 1 The chemical structure of deoxyuridine.

Thus both molecules are in the *anti* conformation. A survey of nucleosides and nucleotides shows that the preferred conformations are such that in general the φ_{CN} value is larger for a C(2') endo pucker than for a C(3') endo pucker^{1,2,4}. The values of φ_{CN} in deoxyuridine, however, are smaller than those found previously for C(2') endo puckers and indicate that the preferred ranges of φ_{CN} values overlap.

In both molecules the orientation of the C(5')-O(5') bond is *trans* with respect to the C(4')-O(4') bond, and *gauche* with respect to the C(4')-C(3') bond. This *trans-gauche* conformation also occurs in fluorodeoxyuridine⁹ and iodouridine¹⁰, and energetically is just as favourable as the more common *gauche-gauche* conformation¹¹. Indeed, the conformation of the C(5')-O(5') bond in those deoxynucleosides which contain uracil shows the three types of preferred conformations for this bond, as shown in Table 1, which summarizes the conformational parameter in these nucleosides.

Table 1 Conformational Parameters of the Nucleosides

Nucleoside	φ_{CN}	Sugar conformation	C(5')-O(5') conformation
Fluorodeoxyuridine ⁹	-59°	C(2') endo	<i>trans-gauche</i>
Bromodeoxyuridine ¹²	-47°	C(2') endo	<i>gauche-trans</i>
Iododeoxyuridine ¹³	-63°	C(2') endo	<i>gauche-gauche</i>
Thymidine ³	-39°	C(3') exo	<i>gauche-trans</i>
Deoxyuridine I	-28°	C(2') endo	<i>trans-gauche</i>
Deoxyuridine II	-24°	C(2') endo	<i>trans-gauche</i>

We thank Dr P. Tollin, Dr D. W. Young and Mr A. R. I. Munns for discussion and advice, and Mr J. Low for assistance with the data collection. We also thank the Science Research Council for the diffractometer and the Medical Research Council for financial support.

ASADUR RAHMAN
H. R. WILSON

Carnegie Laboratory of Physics,
University of Dundee

Received March 19, 1971.

¹ Sundaralingam, M., *Biopolymers*, **7**, 821 (1969).

² Arnott, S., and Hukins, D. W. L., *Nature*, **224**, 886 (1969).

³ Young, D. W., Tollin, P., and Wilson, H. R., *Acta Cryst.*, **B25**, 1423 (1969).

⁴ Wilson, H. R., *Nature*, **225**, 545 (1970).

⁵ Tollin, P., and Cochran, W., *Acta Cryst.*, **17**, 1322 (1964).

⁶ Tollin, P., *Crystallographic Computing* (edit. by Ahmed, F. R.), **90** (Munksgaard, Copenhagen, 1970).

⁷ Tollin, P., *Acta Cryst.*, **21**, 613 (1966).

⁸ Donohue, J., and Trueblood, K. M., *J. Mol. Biol.*, **2**, 363 (1960).

⁹ Harris, D. R., and MacIntyre, W. M., *Biophys. J.*, **4**, 203 (1964).

¹⁰ Rahman, A., and Wilson, H. R., *Acta Cryst.*, **B26**, 1765 (1970).

¹¹ Wilson, H. R., and Rahman, A., *J. Mol. Biol.*, **56**, 129 (1971).

¹² Iball, J., Morgan, C. H., and Wilson, H. R., *Proc. Roy. Soc., A*, **295**, 320 (1966).

¹³ Camerman, N., and Trotter, J., *Acta Cryst.*, **18**, 203 (1965).

Pole Figures of the Orientation of Apatite in Bones

THE orientation of the apatite and collagen in bone was first considered in this work because of its significance in the possible piezoelectric effect in bone. Bone is a polycrystalline material composed chiefly of apatite and collagen, and crystals of a component must be both piezoelectric and orientated in order to produce an overall piezoelectric effect. Orientation studies are also important for an understanding of the mechanical properties and anisotropic characteristics of bone as a structural material. Biologically, bone is a tissue composed of lamellae, blood, bone cells and fluids in a continual state of change. Within this biological system, however, could be a three dimensional network of apatite and collagen on a much smaller scale, which would maintain the mechanical properties of the bone at a constant level, irrespective of the normal sequence of biological changes. This work shows that such a highly organized three dimensional mesh of apatite crystals does exist, and that the exact structure of the mesh in a particular bone may be related to the function of that bone.

Other workers found that apatite crystals were orientated along the length of long bones. Clark and Iball¹, using the film method, reported that the apatite crystals in rat femur were aligned parallel to the axis of the shaft, and also in a transverse plane, tangential to the bone surface.

In this work, preliminary orientation studies of rat bone were made using the film method. Arcing of the 002 powder ring when the X-ray beam was normal to the length of the bone indicated that some apatite crystals were orientated along the length of the bone. When the X-ray beam was parallel to the length of the bone the patterns seemed inconclusive. A more accurate method of determining orientation was required to determine accurate pole figures which would demonstrate the three dimensional arrangement of apatite crystals in bone. A Schulz texture goniometer was used and the pole figures were plotted by computer. This method was originally developed for orientation studies in polymers².

The pole of a set of planes in a crystal is the point where the normal to the planes intersects an enclosing sphere. A pole figure of a particular set of planes is a stereographic projection of such a sphere, on which equal intensities of all poles are contoured. Thus the peaks on a pole figure represent the directions of maximum distribution of the poles of a set of crystal planes. Suitable planes to use were determined by preliminary film and diffractometer work. Planes with a θ Bragg angle well isolated from other peaks give optimum results. In the case of apatite, 002 is a good reflexion and, as it is a basal plane, it has the further advantage that it is comparatively easy to interpret. The prismatic plane (310) was also used because two reflexions incorporating all three indices are necessary to determine completely the orientation of a crystal.

The intensity of the diffracted beam was measured by a proportional counter and recorded on a chart recorder, and was proportional to the distribution of poles in that direction. To produce a pole figure, 720 intensities were read off the chart and the levels of equal intensity contoured by a computer plotter.

Pole figures of the shaft of a bovine femur and of the frontal bone of a bovine skull are shown in Fig. 1a and b respectively. The orientation of the bone specimen with respect to the pole

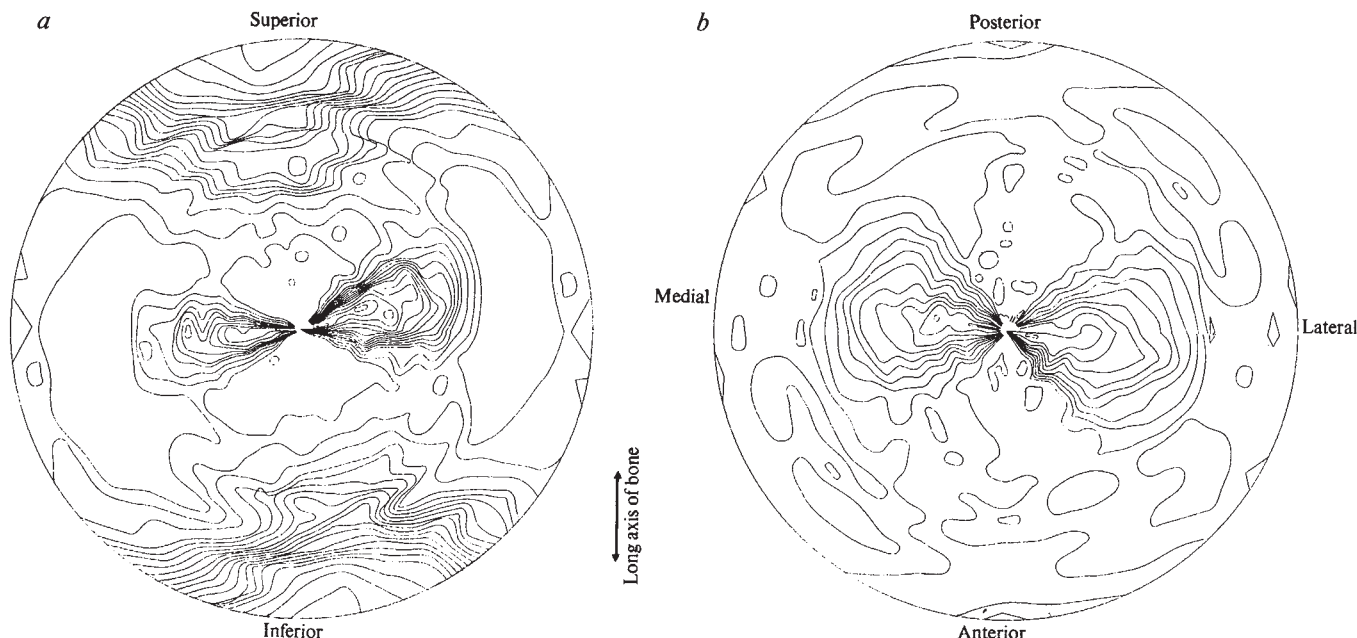


Fig. 1 *a*, 002 pole figure of the apatite crystals in a longitudinal section of the shaft of a bovine femur; *b*, 002 pole figure of the apatite crystals in a tangential section of the frontal bone of a bovine skull.

figure is shown on its periphery. The high peaks of intensity at the north and south poles of the femur 002 pole figure indicate that the long axis of the shaft of the femur is the main direction of orientation. The results also reveal an orientation which is perpendicular to the length of the bone and radial to the long axis. Further, the distribution about this direction is in a characteristic figure-of-eight configuration, which recurs in all the pole figures so far plotted. By contrast, the main orientation direction in the frontal bone of a bovine skull is normal to the surface of the bone. Again, the distribution of intensities about this direction is in the form of a figure-of-eight. There is a considerably smaller distribution in a direction roughly posterior-anterior in the plane of the bone.

Histological slides were prepared from pieces of the femur and skull bones taken as closely as possible to the specimens used for the pole figures. The osteons were orientated along the length of the femur, and tangentially to the surface of the frontal bone of the skull. There is thus a correlation between the osteon orientation and the pole figures for apatite.

In each pole figure the osteon direction is roughly north-south. Thus in the femur the distribution of apatite crystals along the length of the osteons seems to be dominant, but there is also a comparatively strong orientation in a direction perpendicular to the osteon axis. In the skull, the orientation of the apatite is dominantly perpendicular to the osteon axis. This indicates an adaptability of the apatite distribution to the differing mechanical roles of the two bones.

We thank Professor J. M. Zarek for suggesting the topic of this work and for helpful advice, and M. J. Dickens for preparing the histological slides. The work was supported by the Medical Research Council.

J. P. NIGHTINGALE
D. LEWIS

Department of Mechanical Engineering,
University of Surrey,
Guildford,
Surrey

Received March 11, 1971.

¹ Clarke, S. M., and Iball, J., *Prog. Biophys.*, **7**, 226 (1957).

² Lewis, D., Wheeler, E. J., Maddams, W. F., and Preedy, J. E., *J. Appl. Crystallog.*, **4**, 55 (1971).

Non-surgical Assessment of Cardiac Function

CARDIAC catheterization to evaluate myocardial or valvular function should not be used in the seriously ill patient and is difficult in the infant¹. Furthermore, its use is justified for neither periodic screening examinations nor for routine monitoring at surgery. The clinical value of continuous monitoring of a parameter closely related to myocardial efficiency during and immediately after surgery cannot be over-emphasized. The social implications of the ability to screen for valvular disorders and decreased myocardial efficiency before infarction are more difficult to predict.

The close proximity of the mediastinal vessels, especially the aortic arch, to the oesophagus has stimulated the development of an ultrasonic Doppler shift oesophageal probe as a non-surgical technique for estimating aortic arch blood velocity in the conscious patient. The probe consists of an oesophageal tube near the tip of which is inserted a small 'Perspex' mount carrying a piezo-electric disk of 5.0 mm radius. Both faces of the disk are coated with conducting paint and a scratch is made across the diameter of one face, thus electrically separating the crystal into transmitter and receiver. The intact surface on the opposite face forms the common earth electrode. Wires are soldered directly to these three surfaces using low melting point solder (flux 362, alloy, TLC, Multicare Solders Ltd) before passing through the lumen of the oesophageal tube to a Parks 805 flowmeter. Ultrasound is generated continuously by the transmitter part of the crystal which is excited by an rf oscillator to produce a beam of power $\approx 30 \text{ mW cm}^{-2}$. Ultrasonic waves are scattered from moving particles such as blood cells in the path of the beam. The frequency of the back-scattered ultrasound detected by the receiver crystal is Doppler shifted by an amount (Δf) related to the particle velocity (v) by

$$\Delta f = \frac{2fv \cos \phi}{c}$$

where f is the frequency of the emitted ultrasound, ϕ the angle between the ultrasonic beam and the path of the particle and c the velocity of the ultrasound in the intervening tissue.

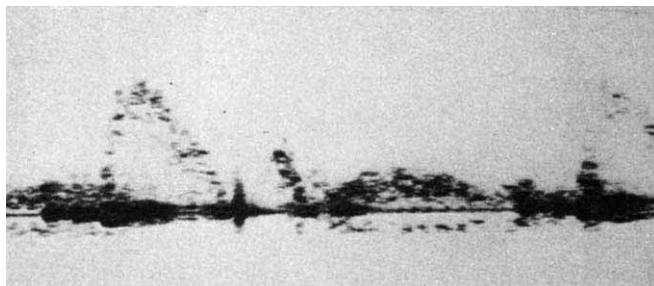


Fig. 1 Spectral analysis of Doppler shift signals from the descending thoracic aorta. The sonagram shows frequency as ordinate against time as abscissa with blackening of trace proportional to amplitude of each frequency component. The second peak represents reverse flow and the high amplitude low frequency components are due to arterial wall movements.

Using an excitation frequency of 5 MHz, the Doppler shift frequencies at each instant are detected as a range of audio signals corresponding to the range of particle velocities in the path of the beam. The problems of analysing such a spectrum of signals have been discussed². To some extent the problems are reduced by the bluntness of the velocity profile in the upper thoracic aorta³, so that the maximum frequency envelope of the sonagram of signals originating from the aortic arch approximates to the mean velocity across the spectrum (Fig. 1).

Alternatively, if the audio signals are fed into a zero-crossing frequency meter, an uncalibrated velocity-time pulse can be displayed on an oscilloscope screen or on a chart recorder. Although the trace is uncalibrated, turbulence caused by a stenotic aortic valve can be detected and percentage regurgitation can be estimated directly from the records. In addition, beat to beat changes in the flow pattern and peak velocity and acceleration can be of considerable value to the surgeon by giving immediate warning of deteriorating cardiac efficiency. The accurate measurement of the flow velocity and its quantitative relationship to myocardial function is the subject of our current work.

The work was supported by a grant (to C. D. S.) from the Medical Research Council, and the Wellcome Trust as part of a general study on pulsatile flow (R. G. G.).

C. D. SIDE
R. G. GOSLING

Department of Physics,
Guy's Hospital Medical School,
London SE1

Received February 18, 1971.

- ¹ Mendel, D., *A Practice of Cardiac Catheterisation*, 5 (Blackwell, Oxford, 1968).
- ² Gosling, R. G., King, D. H., Newman, D. L., and Woodcock, J. P., *J. Ultrasonics*, UIC Papers, 16 (1969).
- ³ Schultz, D., Tunstall-Pedoe, D., Lee, G. de J., Gunning, A., and Bellhouse, B., in *Circulatory and Mass Transport*, CIBA Conf. 172 (Churchill, London, 1969).

Lithium attenuates Drug-induced Hyperactivity in Rats

LITHIUM salts have been used in psychiatry for more than 20 yr, especially for treating manic behaviour¹, and recently there has been a resurgence of interest in these compounds both as a specific treatment of mania and as prophylactics against manic-depressive illnesses in general^{2,3}.

Few systematic behavioural studies, however, seem to have been carried out on animals in controlled experimental situations⁴⁻⁷, possibly because effects on behaviour of non-toxic doses are difficult to demonstrate in 'normal' animals. If rats are given some combinations of dexamphetamine with amyl-

barbitone or chlordiazepoxide they show a characteristic and prolonged hyperactivity in novel environments, which cannot be induced by any dose of the separate drugs^{8,9}. The hyperactivity consists of persistent and apparently stereotyped walking in a maze, and is elicited with great reliability. Because one aspect of mania is increased motor activity, we thought that the hyperactivity induced by the mixture could provide a suitable baseline for testing behavioural effects of lithium in animals. Chronic as well as acute treatments of lithium were given; usually several days are needed for lithium to exert therapeutic effects in manic patients.

We used naive hooded female rats approximately 120 days old at the beginning of the experiments which had been reared in standard conditions of lighting, temperature and humidity and had not been handled since weaning. In chronic experiments rats were injected with either 3 mequiv./kg of lithium chloride (LiCl, isotonic solution), or with the same amount of isotonic saline intraperitoneally daily, always at the same time in the morning, for 15 days. On the fifteenth day, the rats pretreated with LiCl received their last dose (chronic lithium group); those pretreated with saline were divided into two sub-groups: one received its usual morning injection of saline, and the other was given a single injection of 3 mequiv./kg LiCl (acute lithium group). Three hours later all rats were injected subcutaneously with either a mixture of 12.5 mg/kg chlordiazepoxide and 1.18 mg/kg dexamphetamine, dissolved in saline, or with saline (2 ml./kg) alone. Thirty-five minutes after this injection the rats were placed singly in symmetrical Y-shaped mazes, and their behaviour was scored for 5 min, as: (1) the number of times the rats entered the arms of the maze with all four feet across the entrance ('entries'); (2) the number of rears; (3) number of faecal boluses deposited, and the presence or absence of urine in the maze. The observers also rated the ataxia of the rats' walking, using a six point scale. At the end of the Y-maze trial, the rats were anaesthetized with pentobarbitone, blood was withdrawn from the descending aorta, and lithium content was assayed with a flame-photometer (EEL) using a standard method¹⁰.

In further acute experiments the same sequence of operations was followed as that on the last day of the chronic experiment except that: (1) in the morning all rats were injected with 2 mequiv./kg of LiCl (instead of 3 as in the chronic experiments) or the same amount of NaCl; (2) during the second part of the experiment, apart from rats which received subcutaneous injections of saline or of the drug mixture, other groups were given one or other of the constituent drugs, that is, either 12.5

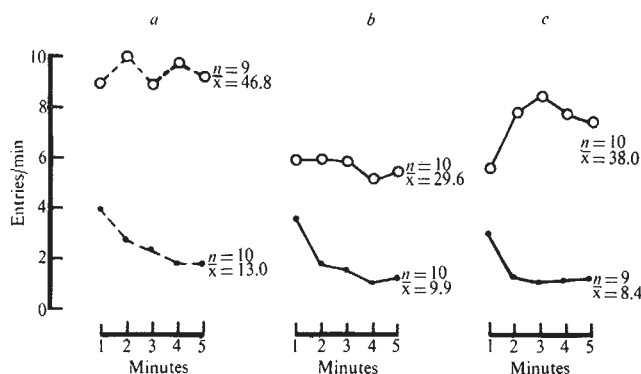


Fig. 1 Effects of acute and chronic LiCl (3 mequiv./kg) on hyperactivity induced in rats by a mixture of 12.5 mg/kg chlordiazepoxide and 1.18 mg/kg dexamphetamine. Group (a) received fifteen daily injections of saline; (b) fourteen injections of saline and one of LiCl; and (c) fifteen injections of LiCl. The rats were tested in a Y-maze either with the mixture (○) or with saline (●). The single injections of LiCl (b) significantly reduced the hyperactivity; chronic pretreatment with LiCl (c) had very little effect. No significant differences were found among saline controls.

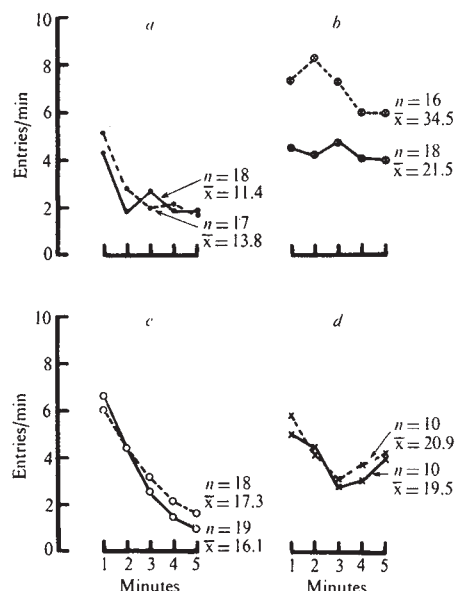


Fig. 2 Effects of a single dose of 2 mequiv./kg LiCl on spontaneous activity of rats under the influence of (a) saline, (b) a mixture of 12.5 mg/kg chlordiazepoxide and 1.18 mg/kg dexamphetamine, (c) 12.5 mg/kg chlordiazepoxide and (d) 1.18 mg/kg dexamphetamine. As in Fig. 1, a single injection of lithium had no significant effects on the number of entries into the arms of a Y-maze by rats unless they were made hyperactive by the drug mixture (b). —, Rats given 2 mequiv./kg isotonic LiCl 3–5 h before testing; ---, rats given the same amount of saline.

mg/kg chlordiazepoxide alone, or 1.18 mg/kg dexamphetamine. Otherwise the same experimental procedures were followed throughout.

In the chronic experiments it was found (Fig. 1) that the animals which had been pretreated with saline for 15 days and had then received the drug mixture showed a near four-fold increase in the number of entries as compared with their own controls ($P < 0.001$, Fig. 1a). The rats pretreated with saline which were given the single dose of LiCl and then the mixture on the test day (Fig. 1b) were less stimulated (Student's *t* test, $P < 0.05$, two tailed). In the chronic lithium group the reduction in the drug mixture-induced hyperactivity was negligible (Fig. 1c). There were no significant differences among the three saline control groups. Thus only acute lithium treatment significantly reduced the hyperactivity.

Because animals tested with 3 mequiv./kg showed some signs of toxicity, for example, diarrhoea, the dose was reduced to 2 mequiv./kg for the next acute experiments (Fig. 2). The mixture again produced a marked, almost three-fold, increase in activity ($P < 0.001$, Fig. 2b) and, as before, lithium treated animals which were tested under the influence of the mixture were less active ($P < 0.01$) than mixture animals pretreated with saline. The single lithium pretreatment did not, however, alter the activity of the saline controls (Fig. 2a). It is clear that acute pretreatment with this dose (2 mequiv./kg) of lithium chloride, as in the previous experiment with 3 mequiv./kg, mitigated though did not completely abolish this particular form of drug-mixture induced hyperactivity; in both cases the number of entries was reduced by approximately a third. The higher levels of activity with the mixture alone in the chronic experiments may have been due to handling; some forms of handling have previously been found to have a similar effect¹¹.

Mixture treated animals were hyperactive in spite of motor incoordination and flaccidity which were reflected in high ataxia ratings made by the observers. Lithium did not increase ataxia ratings in any of the groups. It therefore seems unlikely that the reductions in activity with lithium in the mixture groups can be explained by muscle weakness, although this is frequently reported as a side effect in lithium therapy. As expected, lithium-treated animals urinated more than their controls

during testing although the differences were not statistically significant.

Plasma lithium levels after acute administration of 3 mequiv./kg LiCl were 1.06 ± 0.258 mequiv./l. (mean $\pm$ s.d.) in the mixture and 0.83 ± 0.099 in the saline groups; the corresponding levels in the chronic groups were 0.69 ± 0.166 and 0.84 ± 0.106 . Thus after acute administration plasma levels with the mixture were higher than with saline ($P < 0.05$), and considerably higher than with the mixture after chronic lithium pretreatment ($P < 0.01$).

The mean plasma lithium levels after acute 2 mequiv./kg pretreatment were: 0.49 ± 0.089 (mean $\pm$ s.d.) with saline; 0.52 ± 0.178 with chlordiazepoxide and 0.52 ± 0.144 with dexamphetamine, and 0.67 ± 0.197 with the mixture. The separate drugs did not increase plasma levels but, as before, the levels were increased by the mixture ($P > 0.05$). The results with acute lithium therefore seem to indicate that in the two doses used lithium does not have any marked depressant effect on the spontaneous activity of rats unless the animals have been made hyperactive. Our results are consistent with a study in which lithium inhibited a form of behaviour induced by a combination of desmethylinipramine and Ro 4-1284 (a reserpine-like drug) but it did not inhibit excitation and increased stereotyped behaviour induced by amphetamine alone⁵.

Evidence has been accumulating that acute or short term lithium treatment reduces the amount of noradrenaline available for interaction at receptor sites^{12,13}. The biochemical effects of chlordiazepoxide-dexamphetamine mixtures are not yet established, but in addition to the well known sympathomimetic actions of dexamphetamine, chlordiazepoxide has been found to lower noradrenaline turnover in the brain¹⁴. Thus it is possible that the mixture-induced hyperactivity depends on noradrenergic mechanisms which are affected by acute lithium treatment. This suggestion is supported by the finding that chronic lithium does not markedly alter hyperactivity induced by the mixture nor does it affect noradrenaline turnover in the brain¹⁵⁻¹⁷. Patients have been reported to excrete much less lithium during manic episodes^{18,19}; in our experiments the animals which would have been hyperactive with the drug mixture showed higher concentrations of lithium in the plasma; this may suggest that they also excreted less lithium than animals which were not given the mixture. To that extent results from our acute experiments reflect the clinical situation. In humans, however, lithium usually reduces manic behaviour only after at least several days of treatment, whereas in our experiments chronic lithium was relatively ineffective in mitigating the mixture induced hyperactivity, nor did the mixture increase plasma levels.

We are now testing the effectiveness of lithium against various forms of hyperactivity induced by other means, including by different drugs, by brain lesions and by behavioural manipulations, in order to investigate further the generality of our findings and the possible mechanisms involved.

We thank Drs D. H. Jenkinson and A. V. Juorio and Professors M. Maizels, H. McIlwain and H. O. Schild for valuable comments and discussion and D. U'Prichard, D. I. Carruthers-Jones and Terri Miller for help with experiments. The work was supported by a grant from the National Institute of Mental Health, US Public Health Service, and the Smith Kline and French Foundation. C. C. holds an MRC postgraduate award. P. E. H.-R. holds a University of London postgraduate studentship.

CHRISTINA COX
P. E. HARRISON-READ
HANNAH STEINBERG
M. TOMKIEWICZ

Department of Pharmacology,
University College London,
Gower Street, London, WC1

Received April 17, 1971.

¹ Cade, J. F. J., *Med. J. Austral.*, **36**, 349 (1949).

² Schou, M., *J. Psychiat. Res.*, **6**, 67 (1968).

- ³ Gatozzi, A. A., *US Government Printing Office, National Clearinghouse for Mental Health Information Publication No. 5033* (1970).
- ⁴ Davenport, V. D., *Amer. J. Physiol.*, **163**, 633 (1950).
- ⁵ Matussek, N., and Linsmayer, M., *Life Sci.*, **7**, 371 (1968).
- ⁶ Sheard, M. H., *Nature*, **228**, 284 (1970).
- ⁷ Harrison-Read, P. E., and Steinberg, H., *Nature New Biology*, **232**, 120 (1971).
- ⁸ Rushton, R., and Steinberg, H., *Brit. J. Pharmacol.*, **20**, 145 (1963).
- ⁹ Rushton, R., and Steinberg, H., *Nature*, **211**, 1312 (1966).
- ¹⁰ Helm, H. J. v.d., and Andriess, D., *Clin. Chim. Acta*, **6**, 747 (1961).
- ¹¹ Rushton, R., Steinberg, H., and Tomkiewicz, M., *Nature*, **220**, 885 (1968).
- ¹² Corrodi, H., Fuxe, K., Hokfelt, T., and Schou, M., *Psychopharmacologia*, **11**, 345 (1967).
- ¹³ Schildkraut, J. J., Logue, M. A., and Dodge, G. A., *Psychopharmacologia*, **14**, 135 (1969).
- ¹⁴ Taylor, K. M., and Lavery, R., *Europ. J. Pharmacol.*, **8**, 296 (1969).
- ¹⁵ Corrodi, H., Fuxe, K., and Schou, M., *Life Sci.*, **8**, 643 (1969).
- ¹⁶ Ho, A. K. S., Loh, H. H., Craves, F., Hitzemann, R. J., and Gershon, S., *Europ. J. Pharmacol.*, **10**, 72 (1970).
- ¹⁷ Bliss, E. L., and Ailion, J., *Brain Res.*, **24**, 305 (1970).
- ¹⁸ Trautner, E. M., Morris, R., Noack, C. H., and Gershon, S., *Med. J. Austral.*, **42**, 280 (1955).
- ¹⁹ Gershon, S., and Yuwiler, A., *J. Neuropsychiat.*, **1**, 229 (1960).

Procoagulant Synthesis by Cultured Fibroblasts triggered by Cell Adhesion

SYNTHESIS of a potent procoagulant by cells in tissue culture has been demonstrated but its identity is obscure¹. Activity is low at the beginning of culture of normal human fibroblasts and increases several hundred-fold after 12–24 h of incubation at 37° C. This increase is readily detected by the one-stage assay for factor VIII (antihemophilic globulin) but can also be demonstrated by the two-stage factor VIII assay based on the thromboplastin generation test. Studies in progress to characterize this procoagulant have demonstrated a marked increase of factor XI as well as factor VIII activity and evolution of factor XI activity during contact activation with celite. Increase in activity detectable in the one-stage assay for factor IX and XII was much less. The procoagulant was incapable of clotting a fibrinogen solution and no change in assays for factor V and VII was observed during culture.

These data suggest a relationship of this procoagulant to first-stage coagulation factors, particularly factors VIII and XI. But identification (by *in vitro* coagulation tests) of specific coagulation factors within cells is necessarily complicated by the presence of other procoagulant substances such as phospholipid and tissue factor. Evaluation of the latter is so far incomplete. *In vivo* infusion studies and coagulation factor identification by immunological methods as well as by further *in vitro* testing will be reported in detail elsewhere.

In previous studies considerable variability in procoagulant production was observed between cultures of various cell types¹. Suspension cultures of leucocytes generated procoagulant levels much lower than observed in monolayer cultures. Furthermore, the increase in procoagulant activity in monolayer cultures of several cell types was independent of cell doubling time and paralleled the period during which cells were becoming adherent to the flask. Attention was thus drawn to the process of cell adhesion as a possible triggering mechanism for procoagulant synthesis.

Procoagulant production was studied in cultures of human foreskin fibroblasts prepared in fresh medium at various cell concentrations. Maximum levels were achieved with a cell concentration of 2×10^5 /ml. By contrast, cultures established at a concentration of 4×10^5 /ml. yielded procoagulant levels of approximately 50% of cultures established at the lower cell concentration. Cells in a concentration of 4×10^5 /ml. formed a virtual monolayer from the outset. There was little surface on which the potentially adherent cells could spread.

To investigate further the possible relationship between cell adhesion and procoagulant synthesis, cell cultures were prepared in a concentration of 2×10^5 /ml. in disposable plastic culture flasks (Falcon) and either allowed to remain at rest or placed on a mechanical shaking device to prevent cell adhesion. All cultures were carried out at 37° C. The tilt-top mixer (Lab-Tek aliquot shakers, model 4651, Ames Co., Elkhart, Indiana) rotated through an angle of 80 degrees at a frequency of 20 c.p.m. Periodic observation of cultures by inverted microscopy revealed virtually complete adherence of cells in stationary culture after 4–6 h of incubation. Thereafter rare cells were observed in suspension and haemocytometer cell counts performed on the medium were virtually zero. Conversely, in agitated cultures few cells became adherent. Cultures removed from the mixing device after 12 h of incubation and placed at rest promptly formed monolayers and were indistinguishable from initially stationary cultures. The number of cells declined after 24 h on the shaker, resulting in a less dense monolayer when culture flasks were placed at rest.

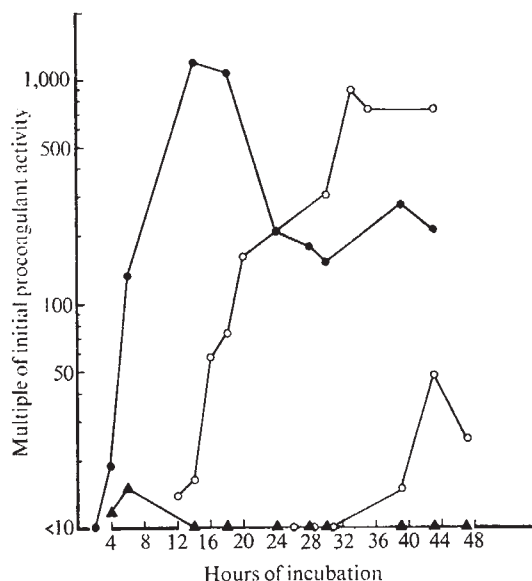


Fig. 1 Effect of shaking on procoagulant production by cultured fibroblasts. ●, Stationary cultures; ▲, cultures incubated on shaker; ○, cultures removed from shaker at 12 and 24 h.

Cells in stationary and rotated flasks were collected at intervals; the plastic flask was opened with a heating element and the monolayer was mechanically dislodged from the floor of the flask. Cell clumps were broken and cells were distributed evenly in the overlying medium by repeated passage through a Pasteur pipette and finally frozen and thawed three times in preparation for analysis. The one-stage assay for factor VIII based on the activated partial thromboplastin time was used in these experiments because it is a sensitive indicator of procoagulant production. Results of assays are presented in Fig. 1. In each of seven experiments the increase of procoagulant activity was prompt and dramatic in stationary cultures. By contrast, cultures incubated on the mixing device failed to generate procoagulant activity. Cultures removed from the mixer after 12 h of incubation and placed at rest promptly began to synthesize procoagulant as cells were permitted to become adherent to the floor of the flask. Ultimate levels equalled those achieved in initially stationary cultures. Cultures agitated for 24 h retained a definite but lesser ability to generate procoagulant when removed from the shaker and placed at rest.

These experiments demonstrate a correlation between cell adhesion and synthesis of procoagulant which is potentially important in elucidating the process of haemostasis regardless of whether the procoagulant is a specific coagulation factor,

for example, factor VIII, or tissue thromboplastin. Local endothelial damage might lead to a number of surface-related events which could enhance blood coagulation locally. For example, the making and breaking of cell surface contacts are necessarily involved in cell infiltration, phagocytosis, migration and proliferation.

The mechanism by which adhesion triggers procoagulant production is obscure. The pertinent cell membrane receptor or metabolic activity responsible for procoagulant synthesis is not defined. It is unlikely, however, that the *in vivo* counterpart of this phenomenon (if such exists) would be restricted to fibroblasts. We postulate that many cell types may synthesize procoagulant *in vivo* and that such synthesis may be mediated by adhesive events occurring at cell surfaces. Perhaps enhanced adhesive activity is the common denominator uniting the host of pathological states characterized by elevated circulating levels of factor VIII. Furthermore, the association of diminished levels of factor VIII and a defect in platelet adhesiveness is well known in von Willebrand's disease. This raises the possibility that the low level of factor VIII observed in this disease is the result of a defective triggering mechanism. Alternatively, the chemical configuration on the cell which permits adhesion may be identical to factor VIII.

LEO R. ZACHARSKI

Research Service,
Veterans Administration Center,
White River Junction,
Vermont

O. ROSS MCINTYRE

Department of Medicine,
Dartmouth Medical School,
Hanover,
New Hampshire

Received January 4; revised March 17, 1971.

¹ Zacharski, L. R., Bowie, E. J. W., Titus, J. L., and Owen, C. A., *Mayo Clin. Proc.*, **44**, 784 (1969).

Mode of Action of Pollen in Breaking Resistance of *Vicia faba* to *Botrytis cinerea*

THE discovery that pollen can transform the mildly virulent fungus *Botrytis cinerea* into a destructive pathogen of broad bean and strawberry¹ poses fundamental and practical problems. One concerns the nature of the stimulants from pollen¹ because *B. cinerea* is normally unable to spread from limited lesions in broad bean leaves even in the presence of fungal nutrients². Another is the mode of action of the stimulants, and particularly their interaction with wyerone acid metabolism. Wyerone acid³ has been identified as an antifungal product of infected bean (*Vicia faba*), and its formation, activity and degradation have been suggested as important factors controlling development of *Botrytis* in *Vicia*^{3,5}. If pollen extracts prevent accumulation of wyerone acid or render the fungus insensitive to the acid, support would be provided for this suggestion.

A suspension of 2×10^6 *V. faba* pollen grains/ml. of sterile distilled water was frozen at -20°C , thawed and shaken for 15 min at 21°C . This procedure was repeated and after centrifugation the supernatant was dialysed for 12 h against sterile water at 5°C . The dialysate was concentrated by rotary evaporation to the original volume of suspension. This pollen extract (pH 5.3) was stored at -20°C . Drops (10 μl .) of suspension of 5×10^5 conidia of *B. cinerea*/ml. sterile water or

pollen extract were placed on upper surfaces of detached bean leaves. Within 5 days at 17°C in moist chambers, conidia in pollen extract caused complete degradation of the leaves, whereas those in water caused some brown flecking beneath infection droplets. From similar leaves, 5 mm diameter disks bearing infection sites were excised 82 h after inoculation, and wyerone acid was extracted, separated and estimated by solvent partition, paper chromatography and ultraviolet spectrophotometry³. Green disks bearing brown lesions caused by spores in water contained 56 μg of wyerone acid/g fresh weight. Black disks from spreading lesions caused by spores in pollen extract contained 97 μg /g fresh weight, so pollen extract had not prevented accumulation of wyerone acid.

Table 1 Germination and Growth of *Botrytis cinerea* in Presence and Absence of 20 $\mu\text{g}/\text{ml}$. Wyerone Acid

Test solution	Germination %	Germ-tube length (μm)
Water alone	95	50–100
Bean extract	100	500–600
Bean extract + wyerone acid	0	0
Pollen extract	100	500–600
Pollen extract + wyerone acid	100	500–600

Spores of *B. cinerea* were suspended ($10^5/\text{ml}$.) in water alone, and in pollen and bean⁴ extracts in the presence and absence of 20 $\mu\text{g}/\text{ml}$. wyerone acid. Droplets (20 μl .) of suspensions were incubated on slides for 20 h at 20°C . Results (Table 1) show that bean and pollen extracts are stimulatory to germ tube growth in the absence of wyerone acid, but that pollen extract also apparently renders spores insensitive to wyerone acid. Separate tests showed that wyerone acid was not lost during incubation with pollen extract. The lowest concentration of wyerone acid which prevented all spore germination in pollen extract was between 500 and 1,000 $\mu\text{g}/\text{ml}$. This contrasts with the concentration of 18 $\mu\text{g}/\text{ml}$. which had a similar effect in bean extract³.

We conclude that pollen enables *B. cinerea* to overcome the inhibitory action of wyerone acid in infected broad bean, and that wyerone acid is an important factor in normal lesion limitation. This raises questions about the mode of action of pollen in stimulating infections of strawberry fruit¹, rye leaves⁶ and wheat heads⁷, and also about normal mechanisms of resistance in these plants.

J. W. M. acknowledges the receipt of a research studentship from the Ministry of Agriculture.

J. W. MANSFIELD
B. J. DEVERALL *

Department of Botany,
Imperial College,
London SW7

Received December 11, 1970; revised March 18, 1971.

* Present address: ARC Unit, Wye College (University of London), Ashford, Kent.

¹ Chou, M. C., and Preece, T. F., *Ann. Appl. Biol.*, **62**, 11 (1968).

² Purkayastha, R. P., and Deverall, B. J., *Ann. Appl. Biol.*, **56**, 139 (1965).

³ Letcher, R. M., Widdowson, D. A., Deverall, B. J., and Mansfield, J. W., *Phytochemistry*, **9**, 249 (1970).

⁴ Deverall, B. J., *Ann. Appl. Biol.*, **59**, 375 (1967).

⁵ Deverall, B. J., and Vessey, J. C., *Ann. Appl. Biol.*, **63**, 449 (1969).

⁶ Fokkema, N. J., *Neth. J. Plant. Pathol.*, **74**, 159 (1968).

⁷ Strange, R. N., and Smith, H., *J. Gen. Microbiol.*, **59**, ii (1969).

Coated Microvesicles in Neurosecretory Terminals of Posterior Pituitary Glands shed their Coats to become Smooth "Synaptic" Vesicles

We have suggested that microvesicles ("synaptic vesicles") within neurosecretory terminals of posterior pituitary glands are by-products of exocytosis formed by micropinocytosis-like activity serving to recapture excess membrane, of neurosecretory granules, incorporated in the cell surface. Our view arose from the discovery of exocytotic figures in electron micrographs of these terminals and the presence, in some such figures, of indications of membrane vesiculation: caveolae with microvesicles of similar size near them^{1,2}. The suggestion harmonized not only with the theoretical requirement for membrane recapture after exocytosis but with the familiar characteristics of the "synaptic vesicle" population: localization in terminal regions where hormone is released; aggregates against the cell membrane, the so called "synaptoid" formations; and increased number after stimulation. The scheme, however, was not entirely satisfactory since classical "synaptic vesicles"³ have smooth membranes while those apparently formed by membrane vesiculation were often seen to be coated, as were the adjacent caveolae². While this coating supported the interpretation of such figures as micropinocytosis-like activity (coating is believed to represent the mechanism inducing membrane vesiculation^{4,5}) it required us to postulate that coated vesicles are somehow transformed into the smooth "synaptic" type². We here present evidence for this transformation.

Neurohypophyses of unanaesthetized rats were exposed following decapitation² and fixed, according to Karnovsky⁶, during electrical stimulation. This yielded many microvesicles with readily discernible coats, some complete but others apparently partial. Such microvesicles can be seen mingled with smooth "synaptic" vesicles and electron-dense neurosecretory granules in Fig. 1. A coated "caveola" is present in the plasmalemma of the fibre toward the left of this illustration.

The appearance of the fully coated vesicle at higher magnification (Fig. 2*A*) conforms to the pattern found in other tissues⁵:

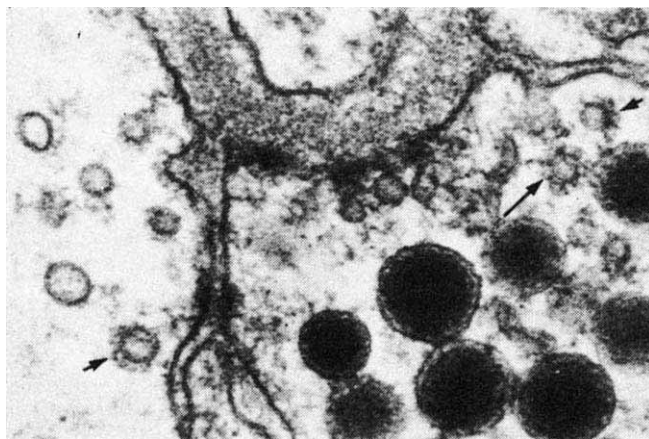


Fig. 1 Neurosecretory terminals in the posterior pituitary gland of the rat fixed during stimulation. The gland, exposed by decapitation, was stimulated electrically (28 V, 1 ms, 20 Hz) through electrodes applied to the stalk and fixed, after 2 min, without stopping stimulation, by flooding with a mixture of glutaraldehyde and paraformaldehyde at room temperature, post-fixed in osmic acid, extracted in block with uranyl acetate according to Karnovsky⁶, and embedded in 'Vestopal'. This is a general view at medium magnification ($\times 86,000$) showing, principally, terminal regions of two neurosecretory fibres abutting basement membrane. The terminals contain a fully coated microvesicle (long arrow), two half-coated microvesicles (shorter arrows), and a number of vesicles with lesser amounts of coating or none at all. A coated caveola suggestive of micropinocytotic activity is evident in the plasmalemma of the terminal toward the left.

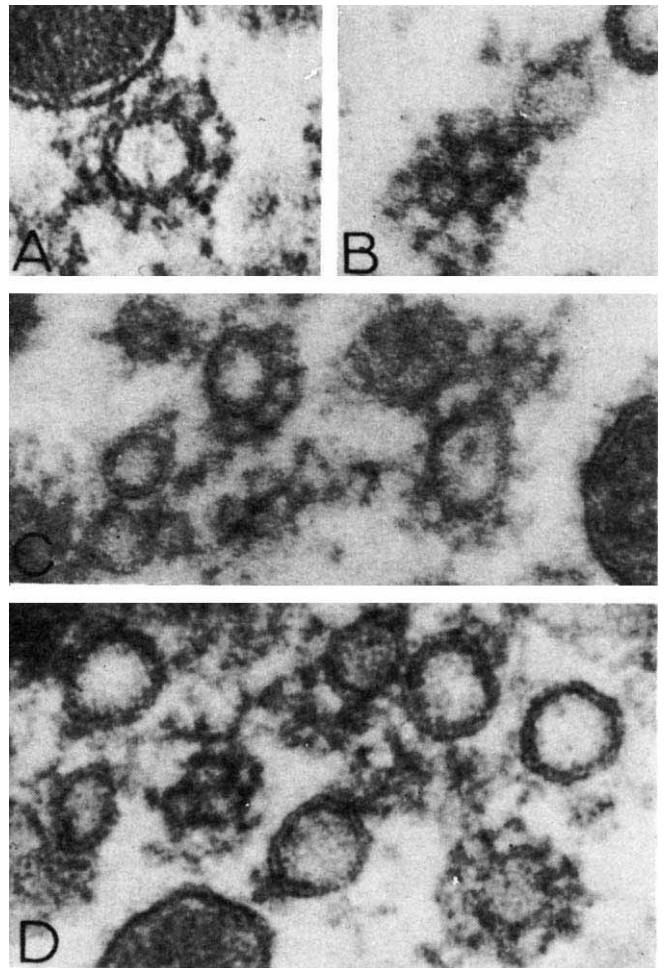


Fig. 2 High magnification ($\times 200,000$) micrographs of neurosecretory terminal regions showing a variety of microvesicles (fully coated, partially coated, and smooth) and shed coats. *A*, A fully coated microvesicle showing the trilaminar structure, radiating "bristles" and dense corona. *B*, A shed coat showing the characteristic alveolar form. *C* and *D*, Fields showing microvesicles with various amounts of coating ranging from full (*D*, lower right) through partial, but still substantial (*C*, the two larger microvesicles), to vestigial, Y-shaped (*D*, lower centre). Note also alveolate images of shed coats and some apparently smooth microvesicles.

a trilaminar vesicle resembling the smooth "synaptic" vesicle except for an investment of rather geometrical appearance suggestive of the spokes of a wheel or a corona of radiating bristles, commonly about ten in number, whose apices are joined and interstices filled by fainter electron densities. From their analysis of corresponding images from conventional neurones Kanaseki and Kadota⁵ suggested they result from the superimposition of the walls of a honeycomb structure (or "basket") investing the vesicle.

Examples of partially coated and smooth vesicles at equally high magnification are presented in Fig. 2*C* and *D*. Scattered among them are faint alveolate figures in which no vesicle can be discerned. These were abundant in our material and with practice easily recognized. Their appearance, in favourable sections (Fig. 2*B*), corresponds closely with that depicted by Kanaseki and Kadota⁵ and, following these authors, we take them to be shed coats. The model of the coated vesicle constructed by Kanaseki and Kadota has a surface composed of twelve pentagons and twenty hexagons. It follows that the figure depicted in Fig. 2*B*, with about ten alveolae, represents a large area of coating. Shedding of such large pieces would result in partially-coated vesicles with extensive denuded areas, but coating could be shed in smaller or larger pieces. Still greater loss of coating, by whatever means, seems likely to

account for vesicles with only a few spokes or bristles (Fig. 2D, centre), and shedding of these vestiges would yield smooth vesicles. The sequence explains how smooth "synaptic" vesicles, devoid of the coating stigmatic of membrane vesiculation^{4,5}, can yet be of micropinocytotic origin.

Although coated vesicles are abundant in our material, it is understandable that they have hitherto received so little attention² in this much studied gland, for coats are not prominent without special staining such as uranyl extraction in block, and when reduced to a few spikes are inconspicuous although often recognizable by characteristic thorn shapes or Y shapes (Fig. 2D, centre). Moreover, the relative abundance of coated forms in glands fixed during stimulation suggests they are short-lived and refractory to capture by conventional methods.

The presence of so many coated vesicles in glands fixed during stimulation considered along with the evidence they shed their coats to become smooth "synaptic" vesicles, greatly strengthens our hypothesis on the micropinocytotic origin of these long-enigmatic structures. Further support of a quite different sort is provided in the following companion report (see next communication⁷).

This work was supported by grants from the US Public Health Service.

W. W. DOUGLAS
J. NAGASAWA
R. A. SCHULZ

Department of Pharmacology,
Yale University School of Medicine,
New Haven, Connecticut 06510

Received April 6; revised June 22, 1971.

¹ Nagasawa, J., Douglas, W. W., and Schulz, R. A., *Nature*, **227**, 407 (1970).

² Douglas, W. W., Nagasawa, J., and Schulz, R. A., *Mem. Soc. Endocrinol.* (in the press).

³ Palay, S. L., in *Ultrastructure and Cellular Chemistry of Neural Tissue* (edit. by Waelsch, H.), 31 (Hoeber, New York, 1957).

⁴ Roth, T. F., and Porter, K. R., *J. Cell. Biol.*, **20**, 313 (1964).

⁵ Kanaseki, T., and Kadota, K., *J. Cell. Biol.*, **42**, 202 (1969).

⁶ Karnovsky, M. H., *J. Cell. Biol.*, **35**, 213 (1967).

⁷ Nagasawa, J., Douglas, W. W., and Schulz, R. A., *Nature*, **232**, 341 (1971).

Micropinocytotic Origin of Coated and Smooth Microvesicles ("Synaptic Vesicles") in Neurosecretory Terminals of Posterior Pituitary Glands demonstrated by Incorporation of Horseradish Peroxidase

ELECTRON microscopic evidence¹⁻³ has led us to believe that smooth microvesicles ("synaptic vesicles") within neurosecretory terminals of posterior pituitary glands arise from coated microvesicles formed by invagination and vesiculation of the cell surface following exocytosis (see preceding communication³).

This view implies that microvesicles of both sorts have been open, at some time, to the extracellular environment, and it should be possible to test this by determining whether they engulf marker substances that do not traverse the intact cell membrane. One such substance, horseradish peroxidase, has been used more than any other to demonstrate micropinocytosis in various cells⁴⁻⁶. We here report the presence of horseradish peroxidase in both coated and smooth "synaptic" microvesicles of neurohypophyseal terminals of rats and hamsters (the species in which exocytosis has been demonstrated^{1,2}) after injecting this substance intravenously or introducing it to isolated glands incubated with excess K to induce secretion⁷.

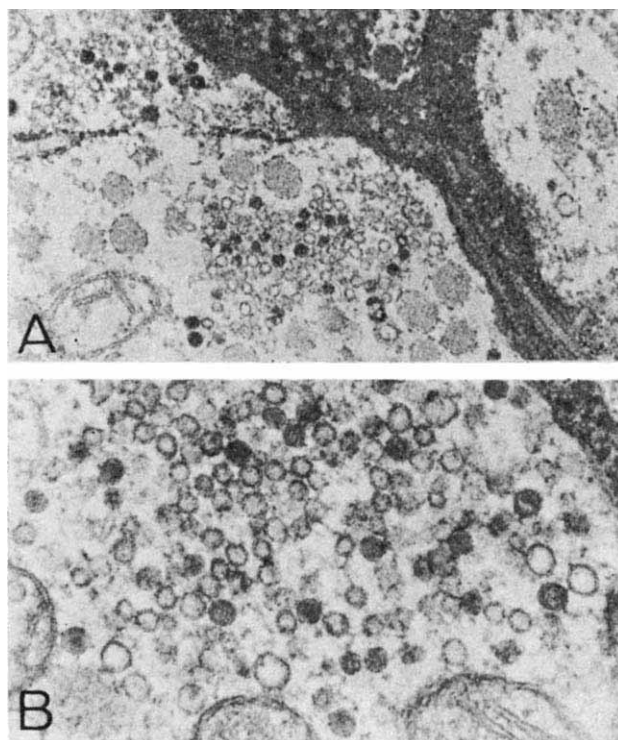


Fig. 1 Incorporation of horseradish peroxidase (HRP) into microvesicles within neurosecretory terminals of posterior pituitary glands of hamster and rat. *A*, Hamster (♂, 90 g), urethane anaesthesia (0.3 ml, 33% w/v given intraperitoneally). HRP 100 mg in 1 ml. Locke by jugular vein. Animal decapitated 10 min later and posterior pituitary fixed with a mixture of glutaraldehyde (3.4%) and paraformaldehyde (1%) in cacodylate buffer and processed for demonstration of HRP according to Karnovsky⁴. Note electron-dense reaction product indicative of HRP is present in numerous microvesicles as well as perineuronal spaces ($\times 38,000$). *B*, Rat (♂, 190 g), unanaesthetized. The posterior pituitary was removed after decapitation and incubated in "high K Locke's solution" containing HRP (100 mg/ml.) for 15 min at 37° C. Many microvesicles contain electron-dense reaction product ($\times 60,000$).

The presence of horseradish peroxidase in many members of the microvesicle population in the two species is illustrated in Fig. 1 where the electron density of the reaction product has rendered these vesicles dark. No reaction product was found in microvesicles in control experiments where horseradish peroxidase was omitted. High power micrographs show most of the labelled microvesicles are smooth "synaptic vesicles" (Fig. 2E), but others are coated (Fig. 2A-D). As noted before², the coated vesicles commonly lie close under the plasmalemma in association with coated caveolae. This, taken together with the incorporation of horseradish peroxidase, leaves little doubt they are the immediate product of membrane vesiculation. The presence of horseradish peroxidase in smooth microvesicles establishes that they too are of micropinocytotic origin. But they are unlikely to arise directly by plasmalemmal vesiculation since coating is believed to be essential for the membrane buckling inducing vesiculation^{8,9} and no caveolae were observed without coating in these or previous experiments³ employing uranyl extraction which renders coats readily visible. The smooth vesicles more probably arise from the coated forms by the shedding of coats described in the preceding report³.

With the micropinocytotic origin of many coated and smooth microvesicles now established, there remain two important questions. First: do all microvesicles in neurohypophyseal terminals arise in this way? This is feasible, for each exocytotic event involving one neurosecretory granule would require formation of some ten to twenty-five microvesicles if the cell surface is not to expand^{1,2}. Failure to detect reaction product

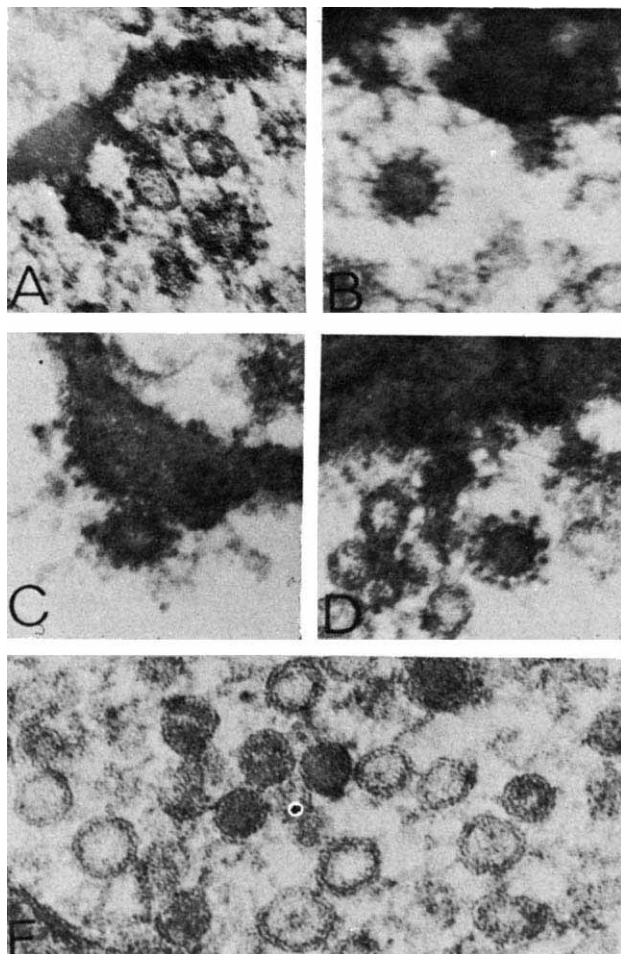


Fig. 2 Terminal portions of neurosecretory fibres showing evidence of micropinocytosis; coated caveolae and adjacent coated microvesicles containing horseradish peroxidase (HRP). (All $\times 128,000$.) *A* and *B*, Hamsters. Pituitaries were removed and fixed 5 min after HRP (100 mg) had been given intravenously. Note coated caveolae in plasmalemma in both, and, in *B*, a coated microvesicle containing reaction product. *C* and *D*, Rats. Pituitaries were incubated *in vitro* (15 and 2.5 min respectively) in high K Locke containing HRP (100 mg/ml.). In *D*, a caveolus and adjacent coated microvesicle is filled with reaction product. In *C*, a coated microvesicle filled with reaction product lies close under a coated depression in the plasmalemma. This may correspond with the images previously interpreted^{1,2} as micropinocytosis occurring within an exocytotic pit. *E*, Horseradish peroxidase in smooth microvesicles of the classical "synaptic vesicle" type. Same preparation as *1B* but higher magnification ($\times 128,000$).

in many microvesicles does not necessarily mean their origin is different: some were probably present before horseradish peroxidase was introduced, and others could have formed without admitting this bulky molecule; moreover, it is unlikely that histochemical detection is 100% efficient. Nevertheless, it is evident that alternative suggestions^{10,11} concerning the origin and significance of microvesicles could still apply to some of the population.

The second question concerns the fate of the microvesicles. To this we can only repeat our previous conjecture that they are disposed of somehow, possibly by lysosomes². They are not likely to be re-used for packaging of hormone since this takes place in the distant perikarya and not in the neurosecretory terminals¹². But, as we pointed out at a symposium in Bristol last year², a similar tightly coupled exocytosis-micropinocytosis sequence could be of functional significance in ordinary nerve endings where local synthesis and packaging (of neurohumour) do occur¹³ and allow for recycling of true synaptic vesicles. Support for this conjecture has since been provided by Gray and Willis¹⁴ who, like us³, have been inspired by Kanaseki and

Kadota's work⁹ and have obtained evidence, in ordinary nerves, that coated vesicles, probably of micropinocytotic origin, can give rise to smooth synaptic vesicles. But clearly our "recycling" hypothesis could only apply if neurohumours, like the neurohormones, are released by exocytosis. Electron microscopy has left this question unanswered since in ordinary nerve endings the caveolae, devoid of electron-dense material, could as well represent micropinocytosis as exocytosis⁶. Nevertheless, some electrophysiological¹⁵ and biochemical¹³ evidence favours exocytosis, and from ontogenetic and ionic parallels it has been argued that secretory mechanisms in ordinary neurones and neurosecretory fibres are probably similar¹⁶.

This work was supported by grants from the US Public Health Service.

J. NAGASAWA
W. W. DOUGLAS
R. A. SCHULZ

*Department of Pharmacology,
Yale University School of Medicine,
New Haven, Connecticut 06510*

Received April 6; revised June 22, 1971.

- ¹ Nagasawa, J., Douglas, W. W., and Schulz, R. A., *Nature*, **227**, 407 (1970).
- ² Douglas, W. W., Nagasawa, J., and Schulz, R. A., *Mem. Soc. Endocrinol.* (in the press).
- ³ Douglas, W. W., Nagasawa, J., and Schulz, R. A., *Nature*, **232**, 340 (1971).
- ⁴ Karnovsky, M. J., *J. Cell. Biol.*, **35**, 213 (1967).
- ⁵ Friend, D. S., and Farquhar, M. G., *J. Cell. Biol.*, **35**, 357 (1967).
- ⁶ Zacks, S. I., and Saito, A., *J. Histochem. Cytochem.*, **17**, 161 (1969).
- ⁷ Douglas, W. W., and Poisner, A. M., *J. Physiol.*, **172**, 1 (1964).
- ⁸ Roth, T. F., and Porter, K. R., *J. Cell. Biol.*, **20**, 313 (1964).
- ⁹ Kanaseki, T., and Kadota, K., *J. Cell. Biol.*, **42**, 202 (1969).
- ¹⁰ Bargmann, W., in *Handbuch der Experimentellen Pharmacologie* (edit. by Berde, B.), 1 (Springer, Berlin, 1969).
- ¹¹ Herlant, M., in *Neurosecretion* (edit. by Stutinsky, F.), 20 (Springer, Berlin, 1966).
- ¹² Sachs, H., in *Handbook of Neurochemistry*, **4**, 373 (Plenum Press, New York, 1970).
- ¹³ Geffen, L. B., and Livett, B. G., *Physiol. Rev.*, **51**, 98 (1971).
- ¹⁴ Gray, E. G., and Willis, R. A., *Brain Res.*, **24**, 14 (1970).
- ¹⁵ Katz, B., *The Release of Neural Transmitter Substances* (Thomas, Springfield, Illinois, 1969).
- ¹⁶ Douglas, W. W., *Brit. J. Pharmacol.*, **34**, 451 (1968).

Changes in ATP and Creatine Phosphate Storage in Skeletal Muscle of Rats trained at 900 and 7,600 Feet

THE hydrolysis of ATP provides the energy source for muscular contraction. ATP is either stored in the muscle or produced by phosphorylation of ADP by creatine phosphate or glycolytic processes, or through oxidative processes in the mitochondria. The processes of adaptation which facilitate the glycolytic or oxidative production of ATP have been studied in trained and acutely exercised animals. After work has been done by the muscle, the oxidative¹ and glycolytic^{2,3} capacities of its fibres increase. Christensen⁴ has shown that neither glycolysis nor oxidative processes take place during short anaerobic bursts of activity. The energy for such limited activities is provided by the hydrolysis of stored ATP and phosphorylation of ADP by creatine phosphate by the Lohman reaction. Previous exercise studies using endurance types of training have not shown any adaptation of either ATP or creatine phosphate⁵⁻⁷, although electrical stimulation⁸ and dwelling at high altitude⁹ have produced increases in creatine phosphate.

Seventy Sprague-Dawley albino rats, 35 days old, were tested for their ability to run on a motor driven treadmill. Fifty-four animals were selected and placed into six groups of nine rats each. Three groups, sedentary, sprint trained and endurance trained, each lived in large dormitory cages, 28 inches by 13 inches by 8 inches, kept in ambient conditions

900 feet above sea level. Three other groups were housed in altitude cages of equal dimensions. These cages were maintained at a simulated altitude of 7,600 feet. The sprint and endurance trained rats at simulated altitude ran on a treadmill housed in a hypoxic atmosphere at ambient pressures, such that the inhaled oxygen pressure was equivalent to that at 7,600 feet. All the groups were maintained for 63 days.

Endurance trained animals were run with progressively increased work loads until they were running for 30 min at an average speed of 34 m min⁻¹ and 30 m min⁻¹ in the ambient and altitude conditions respectively. Throughout the training period the altitude endurance group ran at about 88% of the ambient group's speed. Sprint trained rats were all run at identical speeds regardless of the altitude condition. These rats were run for six to eight intervals of 15 s each with 1 min of rest between each interval. For the final 3 weeks of the study all rats ran at 81.1 m min⁻¹. The rats were run daily; they were run six days per week, beginning with the thirty-fifth day.

At the completion of the training period the rats were anaesthetized with sodium pentobarbitol, and the gastrocnemius-plantaris (as one unit) and the soleus were removed. The muscles were immediately frozen in isopentane chilled to liquid nitrogen temperature. All of the muscles were pulverized in a cold room, deproteinized in cold perchloric acid (30 ml./0.5 g frozen tissue) and analysed for ATP and creatine phosphate¹⁰. Data were analysed using a two-way analysis of variance for groups with uneven *n* values¹¹. The Scheffe *post hoc* test¹² was used to determine significant differences between means.

Table 1 Molar Quantities of Measured Phosphate Compounds of the Soleus

	ATP	Creatine phosphate
Ambient control <i>N</i> =8	2.48	6.50
Ambient sprint <i>N</i> =9	2.15	5.03
Ambient endurance <i>N</i> =4	2.61	7.18
Altitude control <i>N</i> =9	2.28	6.28
Altitude sprint <i>N</i> =7	2.58	6.04
Altitude endurance <i>N</i> =8	2.20	5.99

Quantities in $\mu\text{mol/g}$ of tissue (wet).

Table 2 Molar Quantities of Measured Phosphate Compounds of Gastrocnemius-Plantaris

	ATP	Creatine phosphate
Ambient control <i>N</i> =8	3.13 $\pm$ 0.638	7.89 $\pm$ 0.589
Ambient sprint <i>N</i> =9	3.77 $\pm$ 0.754	8.60 $\pm$ 2.104 *
Ambient endurance <i>N</i> =3	3.45 $\pm$ 1.006	7.49 $\pm$ 1.966
Altitude control <i>N</i> =9	3.51 $\pm$ 0.484	8.59 $\pm$ 1.561
Altitude sprint <i>N</i> =7	3.68 $\pm$ 0.711	10.82 $\pm$ 1.876 †
Altitude endurance <i>N</i> =8	3.64 $\pm$ 1.136	8.78 $\pm$ 1.965 ‡

Quantities in $\mu\text{mol/g}$ of tissue (wet) $\pm$ s.d.

* Greater than ambient endurance ($P < 0.05$).

† Greater than all other groups ($P < 0.05$).

‡ Greater than ambient endurance ($P < 0.05$).

Table 1 summarizes the results of the biochemical analyses of the soleus. Because of the muscle's small size all the soleus from each group was analysed as one sample and no statistical analyses were performed. There was little difference in the quantities of ATP or creatine phosphate between the groups. Table 2 summarizes the values for the gastrocnemius-plantaris. ATP values were not affected by either training or altitude ($P > 0.05$). Sprint training affected creatine phosphate stores.

The ambient sprint group had more creatine phosphate than the ambient endurance group ($P < 0.05$). The altitude sprint group had more creatine phosphate than either the altitude control or endurance groups and also more than the ambient sprint group ($P < 0.05$). Although the two control groups were not different ($P > 0.05$), both of the altitude activity groups had greater quantities of creatine phosphate than their respective ambient counterparts. All of the gastrocnemius-plantaris values exceeded those of the soleus.

Changes in the storage of creatine phosphate did occur as a result of sprint training in the gastrocnemius-plantaris muscles which have a large area of white fibres^{13,14}. Although the low altitude alone had no effect, the increase in creatine phosphate was potentiated by altitude for the running groups. This would suggest that even at 7,600 feet there is a hypoxaemic state which is greater than that even in the sprinting muscle at sea level.

The difference between the soleus and the gastrocnemius-plantaris would be the expected relationship between red and white muscle, red muscle being lower in the high energy phosphates. The fact that the quantities in the soleus were not affected by sprinting would suggest that red muscle is not used during this type of activity. Some fibres of the gastrocnemius-plantaris, however, were affected by endurance running at altitude. It remains to be seen whether the fibres which increased their creatine phosphate stores as a result of altitude endurance training were red, white or intermediate. It is assumed that the white fibres did adapt to short, anaerobic stresses by increasing the quantities of creatine phosphate available for phosphorylation of ADP.

This work was supported by the Wisconsin Heart Association.

JAMES B. GALE

Departments of Health Sciences and Physical Education,
Sonoma State College,
Rohnert Park, California 94928

FRANCIS J. NAGLE

Departments of Physiology and Physical Education,
University of Wisconsin,
Madison, Wisconsin 53706

Received August 27, 1970; revised March 27, 1971.

¹ Holloszy, J. O., *J. Biol. Chem.*, **242**, 2278 (1967).

² Barnard, J., and Peter, J. B., *J. Appl. Physiol.*, **27**, 691 (1969).

³ Peter, J. B., Jefferson, R. N., and Lamb, D. R., *Science*, **160**, 200 (1968).

⁴ Christensen, E. H., Hedman, R., and Salten, B., *Acta Physiol. Scand.*, **50**, 269 (1960).

⁵ Harris, J. W., *Res. Quart.*, **38**, 598 (1967).

⁶ Rulcker, C., Lundberg, P., and Lamek, N., *Acta Vet. Scand.*, **8**, 189 (1967).

⁷ Procter, H. A., and Best, C. H., *Amer. J. Physiol.*, **100**, 506 (1932).

⁸ Palladin, A., and Ferdmann, D., *Z. Physiol. Chem.*, **174**, 284 (1928).

⁹ Tappan, D. V., Reynafarje, D. B., Potter, V., and Hurtado, A., *Amer. J. Physiol.*, **190**, 93 (1957).

¹⁰ Lamprecht, W., and Trautschold, I., in *Methods of Enzymatic Analysis* (edit. by Bergmeyer, H. V.) (Academic Press, New York, 1963).

¹¹ Edwards, A. L., *Experimental Design in Psychological Research* (Holt, Rinehart, and Winston, New York, 1950).

¹² Hays, W. L., *Statistics* (Holt, Rinehart, and Winston, New York, 1963).

¹³ Romanul, F. C. A., *Arch. Neurol.*, **11**, 355 (1964).

¹⁴ Stein, J. M., and Padykula, H. A., *Amer. J. Anat.*, **110**, 103 (1962).

Fertilization of Denuded Rabbit Eggs *in vitro* by Sperm recovered from the Uterus or Vagina

RECENTLY shed eggs are surrounded by a sticky mass of follicular cells known as the cumulus oophorus. The follicular cells, which are closely attached to the zona pellucida and are

arranged in a compact radial pattern, are called the corona radiata. It has been postulated that the follicular cells in the cumulus oophorus can protect the egg against polyspermic fertilization, and can improve the chance of fertilization by providing a large target for the sperm. Further, due to the radial arrangement of the corona radiata, these follicular cells can act in orientating the sperm towards eggs¹. After the dissolution of cumulus oophorus by treatment with hyaluronidase *in vitro*, however, rabbit eggs can be fertilized when transferred into the tubes of mated rabbits^{2,3}. After the dissolution of cumulus oophorus and removal of the corona radiata by manual shaking, the denuded eggs can also be fertilized *in vitro*⁴ or *in vivo*^{5,6}. A comparison of the proportion of eggs fertilized *in vitro* between denuded eggs and those with follicular cells intact has not been made. In addition, it has been speculated that the progesterone present in the follicular fluid or synthesized by the granulosa cells may be necessary for the "acrosome reaction", capacitation of sperm, and the fertilization of eggs *in vitro*⁷. The experiment described here demonstrates (1) that denuded rabbit eggs lacking follicular cells (or progesterone) can be fertilized equally as well as intact eggs with cumulus oophorus, and (2) that sperm recovered from the vagina can fertilize rabbit eggs *in vitro* as well as those recovered from the uterus.

Table 1 Fertilization of Denuded Rabbit Eggs *in vitro* by Sperm recovered from the Uterus or the Vagina

Collection site and No. of sperm (millions/ml.)	Denuded eggs			Eggs with follicular cells intact		
	Total	No. fertilized	Percentage fertilized	Total	No. fertilized	Percentage fertilized
Uterus (0.06–0.17)	48	16	33	92	27	29
Vagina (0.23–4.2)	45	24	53	30	14	47
Ejaculate (0.97–5.1)	41	0	0	32	0	0

Mature female rabbits were injected either subcutaneously or intramuscularly with 170 IU of PMS to induce growth of a large number of follicles and 72 h later they were injected intravenously with 90 IU of HCG to induce ovulation. Approximately 15 h after injection of HCG the Fallopian tubes were removed and flushed with a tissue culture medium (TC 199) to recover the eggs. Immediately after recovery, one group of eggs in the mucous clot was placed in a watch glass containing 0.5 ml. of 0.1% hyaluronidase in TC 199 and the other group was placed in another dish containing no hyaluronidase. After the dissolution of the cumulus (about 10 min), the hyaluronidase-treated eggs were placed in a small test tube (4 cm long) and denuded by being shaken vigorously by hand for about 5 min. The denuded eggs were then placed in another dish containing TC 199. The procedures for fertilizing rabbit eggs are essentially similar to those reported previously⁸.

For the recovery of capacitated sperm, one female was mated consecutively by three fertile bucks. It was anaesthetized with nembutal 14–18 h after mating. After making a medial incision the uterine horn or vagina was flushed with 2–3 ml. of a freshly prepared medium⁹. About 1 ml. of uterine or vaginal washing (containing strongly motile sperm) was placed in a 1.5 ml. Carrel flask. The denuded eggs were placed into one flask prepared in this way, and the intact eggs with cumulus clot were placed into another. The flasks were closed with a rubber bulb and attached to a gentle rocking device in an incubator at 38° C. After 3–4 h the eggs were picked up and subcultured in a 6 ml. capacity Carrel flask containing 4 ml. of

TC 199 incorporating 20% heated rabbit serum. After incubation for another 18–20 h, the eggs were taken out, mounted on a slide and examined. They were then fixed with neutral formalin, stained for the assessment of fertilization^{2,8}, and examined again. Usually a second maturation chromosome pattern or a group of small nuclei can be seen in the unfertilized eggs. Most of the fertilized eggs are at the four-cell stage. Fragmentation of fertilized and especially unfertilized eggs is fairly common.

The results presented in Table 1 indicate that there is no difference in the proportion of eggs fertilized *in vitro* whether the eggs are denuded or not. The presence of follicular cells (or progesterone) does not appear to play a role, therefore, for the fertilization of rabbit eggs *in vitro*. We have also discovered that the proportion of eggs fertilized by vaginal sperm is significantly higher than by uterine sperm. Whether this is due to a higher concentration of sperm or better vitality of sperm in the vaginal washing is still to be determined.

This work was supported by grants from the National Institute of Child Health and Human Development, US Public Health Service, and the Ford Foundation.

M. C. CHANG
A. HANADA
DOROTHY M. HUNT

Worcester Foundation for Experimental Biology,
Shrewsbury,
Massachusetts

Received April 13, 1971.

- ¹ Austin, C. R., *The Mammalian Egg*, 100 (Blackwell Publications, Oxford, 1961).
- ² Chang, M. C., *J. Exp. Zool.*, **121**, 351 (1952).
- ³ Dickmann, Z., *J. Anat.*, **98**, 392 (1964).
- ⁴ Bedford, J. M., and Chang, M. C., *Nature*, **193**, 898 (1962).
- ⁵ Chang, M. C., and Bedford, J. M., *Fert. and Ster.*, **13**, 421 (1962).
- ⁶ Harper, M. J. K., *J. Exp. Zool.*, **173**, 47 (1970).
- ⁷ Edwards, R. G., Steptoe, P. C., and Purdy, J. M., *Nature*, **227**, 1304 (1970).
- ⁸ Chang, M. C., *Nature*, **184**, 466 (1959).
- ⁹ Brackett, B. G., *Schering Symposium on Mechanisms Involved in Conception*, 73 (Pergamon Press, Vieweg, 1970).

Role of Skin and Neurohypophyseal Hormones in the Adaptation of the Toad *Bufo viridis* to High Salinities

MANY people maintain that ADH is important in the control of water and salt metabolism of amphibia¹. The toad *Bufo viridis* is one of the species most resistant to dehydration and high ambient salinity, in which situations it has a high plasma salt concentration. In toads adapted to high salinity, the urine sodium chloride concentration increases to about plasma level, whereas active sodium transport, as measured by short circuit current, is almost completely reduced (ref. 2 and our measurements). The animals apparently do not drink and water is passively transferred along the osmotic gradient from the ambient solution into the body. We found that the sensitivity to added hormone was lost from the skins of animals that were adapted to saline solutions above 400 mosm.

We studied the water flux across isolated portions of skin in saline-adapted animals. The skin was carefully dissected and attached to a 'Perspex' conus, the inside facing out, and the preparation was maintained in aerated Ringer solution (NaCl 120 mmol l.⁻¹; KCl 2.5 mmol l.⁻¹; CaCl₂ 2 mmol l.⁻¹ at pH=8). The inner solution was hypotonic, and water transfer was followed gravimetrically on a 0.1 mg precision Mettler balance. The temperature was maintained at 20°±1° C. A typical experiment is shown in Fig. 1. When skin was used from animals adapted to high saline, osmotic equilibrium was

adjusted with sodium chloride. Synthetic oxytocin ('Syntocinon', Sandoz) was used throughout.

Skin from toads kept in either tap water of 230 mosm solution (containing either sodium chloride or sucrose) was almost impermeable to water; the application of hormone to the external bath (serosal side) stimulated the water flux across the skin. When skin from toads adapted to 400 mosm NaCl solution was tested, water flux occurred as soon as there was a gradient across the skin; the application of hormone to the external bath did not have any further effect.

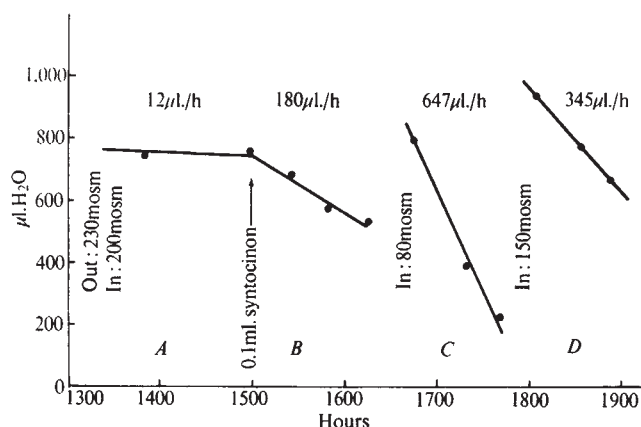


Fig. 1 Results of a typical experiment of the *in vitro* method to follow water flux across the isolated skin. In the first period (A) an osmotic gradient was imposed across the skin, with its inside facing outwards. At the arrow, hormone was added to the external medium and weight change followed. In the two successive periods (C, D) different gradients were imposed. The rate of water flux for each period is indicated above.

Although the solutions used in the latter case were hypertonic to normal Ringer of amphibia, they were definitely not hypertonic to the preparations being studied, which were always in osmotic equilibrium. To check this, however, we tested skin from normal animals in hypertonic solution and skin from 500 mosm NaCl-adapted animals in hypotonic solution. An effect similar to that of ADH was observed in the first case, which was similar to observations³ on frog urinary bladder. In the second case, the change of the solution did not change the properties of the skin, and water transfer ensued without the addition of hormone.

In animals adapted to 400 mosm NaCl and then brought back to tap water through 230 mosm NaCl, the effect was reversible in that the responsiveness of the skin to the hormone was restored.

The increased permeability of the skin to water, which is maintained without the addition of hormone, seems to be very important to the water metabolism of the toad. To ascertain the amount of hormone in the circulation, we used the *in vitro* assay⁴ of hormone on isolated normal bladders. Blood collected from three or four animals was pooled, centrifuged in the cold, and 2 ml. of the plasma was added to 10 ml. of the external solution. No response was observed with plasma from animals that were maintained in either tap water, 230 mosm or 450 mosm NaCl. Hydro-osmotic activity was found, however, in the plasma of animals dehydrated in 2.5% NaCl for 3 h (ref. 5). In another series of experiments the hormonal activity of the hypophysis was investigated semi-quantitatively by using the *in vitro* method with the addition of neurohypophyseal extract. The results showed the existence of almost the same activity in the animals from either tap water, 230 mosm NaCl or 400 mosm NaCl solutions.

It seems that adaptation to high salinity in these toads leads to some fundamental physiological changes in the skin. It is of interest to mention that Dicker and Elliott⁶ working on

Rana crancrivora, a euryhaline species inhabiting brackish mangrove swamps, also found an apparent lack of hydro-osmotic response to neurohypophyseal peptides in the isolated bladder.

We are at present investigating whether the changes in the skin that we found are also reflected at the ultrastructural level.

URI KATZ
JUDITH WEISBERG

Department of Zoology,
Hebrew University of Jerusalem

Received December 14, 1970; revised March 12, 1971.

- ¹ Deyrup, I. J., in *Physiology of the Amphibia* (edit. by Moore, H. J.) (1964).
- ² Gordon, M. S., *J. Exp. Biol.*, **39**, 261 (1962).
- ³ Pipoché, P., Parisi, M., and Bourguet, J., *Biochim. Biophys. Acta*, **193**, 231 (1969).
- ⁴ Bentley, P. J., *J. Endocrinol.*, **17**, 201 (1958).
- ⁵ Bentley, P. J., *J. Endocrinol.*, **43**, 359 (1969).
- ⁶ Dicker, S. E., and Elliott, A. B., *J. Physiol.*, **210**, 137 (1970).

Sterilization of *Aedes aegypti* (L.) as a Result of Chronic Exposure to Low Doses of Beta Radiation

THE application of the sterile male technique for the control of insect populations depends on the release of an adequate number of sterilized males. Whitten and Taylor¹ have suggested that the effectiveness of the method might be enhanced by releasing sterile females along with the sterilized males. In the case of mosquitoes, however, any addition to the number of biting females already present in a native population would be unwelcome.

So far efforts to use the sterilized insect for mosquito population control have generally involved insects that were irradiated during pupal or adult stages. We have succeeded in sterilizing more than 70% of the adults of laboratory populations of *Aedes aegypti* (L.) by exposing them to a total dose of beta radiation estimated to be as small as 90 rad during the early larval stages. This dose is about one-tenth of the X-ray doses which have been used to sterilize the pupal or adult stages². Willard³ has studied the long term effects of acute X-ray irradiation of the egg, larval and pupal stages of *A. aegypti*, on the population dynamics of that species.

If sterilized males and females are to be used for mosquito population control, it is important that a significant number of viable eggs is not laid as a result of their mating. Twelve groups of 100 newly hatched *A. aegypti* larvae were irradiated for 192 h during instars I to IV, by rearing them at $24 \pm 1^\circ \text{C}$ in media containing 0.1, 1.0 and 10 $\mu\text{Ci/ml}$. ^{90}Sr in equilibrium with its daughter product ^{90}Y . The amounts of ^{90}Sr and ^{90}Y accumulated by the larvae were quite variable, so it was not feasible to calculate the dose delivered. The use of sealed $^{90}\text{Sr} + ^{90}\text{Y}$ plaques placed in the rearing medium will deliver only an external dose which can be calculated. Controls were exposed to natural background radiation only. Hatching and rearing methods were essentially those described by Riordan⁴. After 192 h, instar IV larvae were removed from the radioactive media, rinsed and placed in demineralized water to pupate. The adults were allowed to mate. Males were offered a 50% solution of honey in water. Females were also allowed to feed on guinea-pigs. Blood-feeding from guinea-pigs, and egg laying were observed. The effects on egg laying of mating irradiated males with non-irradiated females, and *vice versa*, were not examined in these experiments.

Larvae reared in 10 $\mu\text{Ci } ^{90}\text{Sr}/\text{ml.}$ did not pupate, while those reared in 0.1 and 1.0 $\mu\text{Ci}/\text{ml.}$ developed normally and the adults had no gross external morphological abnormalities. The development of the ovaries and the testes, however, had been arrested.

Table 1 Effect of Continuous Beta Irradiation of Instar I to IV Larvae on Feeding and Egg Viability of Adult *A. aegypti*

^{90}Sr concentration ($\mu\text{Ci}/\text{ml.}$)	Males		Females	
	Took honey-water	Took blood meal	Laid eggs	Viability of eggs laid
Background	Yes	Yes	Yes	80%
0.1	Yes	Yes	No	—
1.0	—†	No	No	—
10.0*	—	—	—	—

* Larvae failed to pupate and died 25 days after moulting to instar IV.

† Not observed.

Feeding and egg laying are summarized in Table 1. All females were dissected 10 days after those in control groups laid eggs, or when they became moribund. Females irradiated as larvae laid no eggs. In addition, females in the 1.0 $\mu\text{Ci } ^{90}\text{Sr}/\text{ml.}$ treatment groups took no blood. Dissection showed that most of the radiation-treated females had motile sperm in their spermathecae, but their ovaries were atrophied. According to Bugher and Taylor⁵, failure to produce eggs is the first indication of abnormality caused by increasing radiation dose. Leahy and Craig⁶ have reported an accessory gland substance, transmitted from the male to the female during copulation, to be a stimulant for oviposition in *A. aegypti* and *A. albopictus*. The irradiated females in our experiments may not have received this substance. Smittle⁷, studying the effects of gamma radiation on *Culex pipiens quinquefasciatus*, suggested that sterilization of the females was the result of inhibition of egg production. Although some of the irradiated females in our experiments took blood (Table 1), their ovaries were atrophied, and therefore eggs could not be produced.

Our observations, that females reared in 1.0 $\mu\text{Ci } ^{90}\text{Sr}/\text{ml.}$ took no blood meals and laid no eggs, suggest that sterile females might usefully be used in combination with sterile males to control mosquito populations. The production of large numbers of larvae⁸, irradiated externally by means of sealed beta sources, would not be difficult. Investigations of the effects of chronic external beta and gamma radiation on mosquito populations are in progress.

J. E. GUTHRIE

Whiteshell Nuclear Research Establishment,
Atomic Energy of Canada Limited,
Pinawa,
Manitoba

R. A. BRUST

Department of Entomology,
University of Manitoba,
Winnipeg,
Manitoba

Received March 4, 1971.

¹ Whitten, M. J., and Taylor, W. C., *J. Econ. Entomol.*, **63**, 269 (1970).

² Willard, W. K., *Radiat. Res.*, **37**, 493 (1969).

³ Willard, W. K., in *Radiation and Terrestrial Ecosystems* (edit. by Hungate, F. P.), *Health Phys.*, **11**, 1577 (1965).

⁴ Riordan, D. F., *Canad. J. Zool.*, **43**, 497 (1965).

⁵ Bugher, J. C., and Taylor, M., *Science*, **110**, 146 (1949).

⁶ Leahy, M. G., and Craig, G. B., *Mosquito News*, **25**, 448 (1965).

⁷ Smittle, B. J., *Florida Entomologist*, **51**, 59 (1968).

⁸ Fay, R. W., McCray, M., and Kilpatrick, J. W., *Mosquito News*, **23**, 210 (1963).

Transmission of *Phytophthora* Pod Rot of Cocoa by Invertebrates

Phytophthora palmivora (Butl.) Butl., the causal agent of black pod of cocoa (*Theobroma cacao* L.), accounts for an estimated 19% crop loss in Ghana¹, and losses are considerably higher in wetter countries such as Nigeria and the Cameroun Republic. The epidemiology of the disease is still uncertain but rain-splash from soil is considered by some workers^{2,3} to trigger-off disease cycles.

During a study of 200 mature cocoa trees, black pod lesions were consistently associated with pods colonized by tent building ants. These tents serve as shelters for coccids (scale insects) which usually feed on the pod peduncle. The coccids are tended by ants which remove excess sugars for their own use. An extensive survey of black pod-ant tent relationships has been undertaken and five stands of mature cocoa trees, comprising ant-free and ant-colonized trees, have been selected for comparative studies. Preliminary results indicate a positive correlation between certain tent building ant species and the levels of black pod. The type of material used in the construction of the tents is the most important factor involved. For this reason ants of the genus *Crematogaster*, and specifically *C. striatula* Emery, appear to be the most important vectors of black pod. These ants remove the outer layers of healthy and diseased pods and use the material to construct tents and other temporary shelters. *C. striatula* in well shaded cocoa can assume complete dominance over wide areas. Black pods left on the ground after harvesting or left hanging on the tree are used as building material and small discrete tents are constructed primarily around the pod peduncle. The ants are, therefore, effectively transferring infected pod material from the ground or from on the tree sources to healthy pods. Ants moving from the base of the tree upwards have been trapped and in several cases material containing spores of *P. palmivora* was being carried for use in tent construction. *C. africana*, *C. clariventris* and *C. depressa*, in addition to *C. striatula*, have all been observed scraping and carrying away material from black pods left hanging on the tree or lying on the ground.

Other ant species seem to be more incidental in the spread of black pod as they rarely use diseased pod material in tent construction. *Camponotus acvapimensis* builds very large tents composed almost entirely of soil. Soil infested with spores of *P. palmivora* may be carried to healthy pods in "soil balls" and ultimately cause infection. *Pheidole* spp., mainly *P. megacephala*, and *Acantholepis capensis* also build large soil tents, although almost any freely available material may be included. The importance of these ants in the dissemination of black pod will depend very much on the amount of viable soil inoculum present in a particular area.

Tent building ant species, therefore, may be important vectors of black pod causing local epiphytotics. In particular, it is thought that *C. striatula* because of its building habits and its complete dominance under certain conditions may be responsible for serious black pod losses. The soil-tent ant species, although less directly involved in the dissemination of diseased material, may initiate disease outbreaks by carrying inoculum in soil to healthy pods and creating primary infection foci; secondary spread by rain-splash may follow. The proportion of cocoa trees colonized by tent building ants may be

very high⁴ and their significance in the vertical spread of black pod should not be overlooked.

It is considered that ants are responsible primarily for vertical disease transmission, but horizontal spread may be attributable to other invertebrate vectors. Thorold⁵ noted the absence of infection gradients in the field and concluded that horizontal dispersion of black pod follows a random pattern of the type expected for an airborne disease. Nevertheless, sporangia of *P. palmivora* have never been isolated from the air^{6,7} and horizontal spread is still speculative and largely unexplained. Microscopic examination of faeces of herbivorous invertebrates commonly found on black pod revealed the presence of *P. palmivora* spores. Faecal material, wound-inoculated into healthy pods, produced typical black pod lesions. Viable spores have now been recorded from the faeces of eight beetle species, three millipede species, four caterpillar species and four snail (slug) species. Beetles are by far the most prevalent invertebrates on black pods and one species in particular (family *Nitidulidae*) occurs on 50–60% of black pods. The invertebrates feed on the outer tissues of black pods and in the process ingest mycelium and spores of *P. palmivora*. These remain intact during passage through the gut and are ejected together with cutin, pod hairs and stomatal guard cells. An extremely high percentage of the sporangia pass through the alimentary tracts of snails, millipedes and caterpillars without damage and pod inoculations from these faeces invariably cause infection. Most sporangia in beetle faeces are devoid of contents and infectivity is low. It is thought that at a certain stage in development the sporangial papilla becomes susceptible to enzyme attack and the sporangial contents are digested by the insect. Chlamydospores seem to remain unchanged after ingestion by invertebrates.

Flying insects, carrying inoculum of *P. palmivora* in the faeces, may explain the random occurrence and "over-dispersion" of black pod recorded by Thorold^{5,8} which led him to suggest air dispersal as a means of dissemination. The importance of the other invertebrates in the transmission of black pod is difficult to assess. But the widespread occurrence of these animals in cocoa farms implies that they may be responsible for some horizontal spread. By feeding on black pods left on the ground after harvesting they may move around large amounts of inoculum, possibly introducing spores in previously disease free soil from where they may be dispersed by ants or rain-splash.

The answer to black pod control may lie partly in the efficient application of insecticides and Weststeijn⁹ noted that the addition of a DDT-formulation to lime Bordeaux mixture distinctly improved the control of black pod. The effective removal of diseased pods from the cocoa farm after harvesting is an obvious control method, thereby limiting the amount of infective material available to tent-building ants and herbivorous invertebrates. This preliminary evidence of the transmission of black pod by invertebrates may partly explain the vertical and horizontal spread of this economically important pathogen.

I would like to thank Mr B. Bolton for initial identification of the ant species. I am a member of the British Research Team of the British Overseas Development Administration.

H. C. EVANS

Cocoa Research Institute (CSIR),
Tafo,
Ghana

Received March 22, 1971.

¹ Blencowe, J. W., and Wharton, A. L., *Rep. 6th Commw. Mycol. Conf. Kew*, 139 (1961).

² Grimaldi, J., *Rep. Cocoa Conf.*, London, 90 (1957).

³ Okaisabor, E. K., *Nigerian Agric. J.*, 2, 67 (1965).

⁴ Strickland, A. H., *Rep. W. Africa Cocoa Res. Inst.*, 1947–48, 33 (1949).

⁵ Thorold, C. A., *Nature*, 170, 718 (1952).

⁶ Wharton, A. L., *Rep. W. Afr. Cocoa Res. Inst.*, 1954–1955, 56 (1955).

⁷ Okaisabor, E. K., *Third Intern. Cocoa Res. Conf., Accra, Ghana*, 1969 (in the press).

⁸ Thorold, C. A., *Trans. Brit. Mycol. Soc.*, 38, 435 (1955).

⁹ Weststeijn, G., *Ann. Rep. Cocoa Res. Inst. Nigeria*, 1966–1967, 75 (1968).

Metabolic Implications of Polymorphism as an Adaptive Strategy

ENZYME polymorphisms are known to be widespread in natural populations^{1–3}. No adequate theory has been advanced to account for their frequent occurrence; some workers support the proposition that they are selectively "neutral"^{4–6}, while others present evidence that they are maintained in natural populations through natural selection rather than by non-selective processes^{1,7–9}.

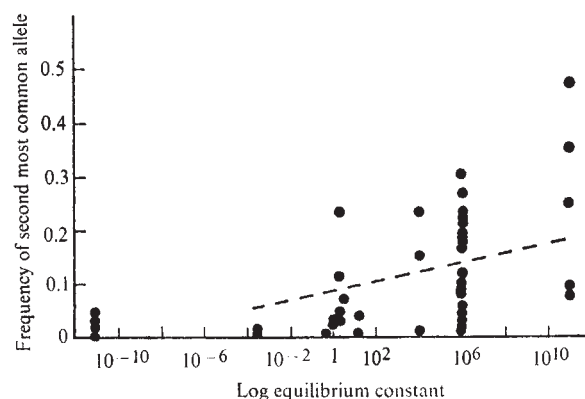


Fig. 1 Relationship of polymorph frequencies to equilibrium constant. For each of fourteen enzymes, the reported frequency of the second most common allele for a given population is plotted as a function of the corresponding equilibrium constant. The coefficient of correlation for a linear regression analysis of these data is 0.483, significant at the 0.1% level. Non-specific enzymes were not analysed. $F_{n-4} = 27.08$, $P \leq 0.005$.

The hypothesis that selection acts to maintain enzyme polymorphisms in natural populations has important metabolic implications not previously considered. Within any metabolic pathway a given catalyst will affect the rate of end-product formation according to its role in the kinetics of the overall sequence; those enzymes which exert acute control over flow through the pathway (that is, irreversible and rate-limiting steps) should be most individually sensitive as sites of action of selective forces (in contrast to selection on generalised metabolic systems¹⁰). Thus if natural selection affects the output of metabolic pathways by modulating the extent of molecular heterogeneity among the enzymes of the pathway, such selection should be most efficient if applied at those metabolic steps which most specifically control flow through the pathway.

The results of applying selection to enzymes with different kinetic roles in metabolism can be seen most clearly in irreversible reactions. In a freely reversible reaction, mass action must strongly dilute the effect of any change in the kinetics of the reaction on previous or subsequent reactions. An irreversible reaction may act by mass action to promote the operation of the pathway both proximal and distal to it, previous reactions being drawn along by its removal of substrate, and subsequent reactions being driven by its products; thus the activity of an enzyme catalysing an irreversible reaction has effects extending over the whole pathway. The action of selection in maintaining

a natural, stable enzyme polymorphism would be enhanced if applied to such an "acute control" point, as it would critically affect the efficient operation of the entire metabolic sequence.

Table 1 Enzyme Polymorphism in *Drosophila*

Enzyme	K_{eq}	Sample size	Populations significantly polymorphic (%)	Index of polymorphism
Alcohol dehydrogenase (1.1.1.1)	1×10^{11} (19)	1,150	100	0.330
Aldehyde oxidase (1.2.3.1)	2×10^6 (20)	850	66	0.120
Xanthine dehydrogenase (1.2.3.2)	Very large (21)	703	66	0.131
α -Glycerophosphate dehydrogenase (1.1.99.5)	1×10^4 (12)	831	40	0.083
Glucose-6-phosphate dehydrogenase (1.1.1.49)	3×10^2 (20)	527	28	0.054
Hexokinase (2.7.1.1)	155 (23)	141	50	0.007
Isocitrate dehydrogenase (1.1.1.41)	20 (20)	743	40	0.029
Malic enzyme (1.1.1.40)	20 (24)	687	20	0.012
Phosphoglucosmutase (2.7.5.1)	17 (25)	464	75	0.137
Fumarase (4.2.1.2)	4 (26)	577	0	0.013
6-Phosphogluconate dehydrogenase (1.1.1.44)	1 (27)	187	0	0
Phosphohexose isomerase (5.3.1.9)	0.3 (28)	251	0	0.002
Aldolase (4.1.2.13)	2×10^{-4} (29)	515	0	0.006
Malate dehydrogenase (1.1.1.37)	7×10^{-13} (20)	1,012	10	0.015

Reported values of the equilibrium constants of the fourteen enzymes are listed; for enzymes with variation in reported values, the value closest to 1.0 was chosen. In all cases, the physiological direction of the reaction in insects was used in determining the equilibrium constant. Also listed for each enzyme is the sample size, n (the total number of individuals from all populations examined for a given enzyme), the percentage of the populations examined in which high frequency polymorphisms (two or more alleles with frequencies $\geq 4\%$) are reported, and the index of polymorphism ($1-P$).

These metabolic considerations predict that if enzyme polymorphisms are maintained in natural populations by selective forces, a survey of the occurrence of polymorphisms in various enzymes of individuals of natural populations would reveal a greater degree of polymorphism in enzymes catalysing physiologically irreversible reactions; significantly less polymorphism would be expected for enzymes whose reactions are freely reversible, or possess equilibria strongly favouring the reverse of the physiologically significant direction.

Unfortunately, most surveys of enzyme polymorphism reported so far cannot adequately test this hypothesis, as they have been conducted on very small samples of natural populations^{1,11-13}. Investigations of inbred laboratory strains¹⁴ are unsatisfactory for this purpose as the selective advantage of polymorphisms in such strains is less clear. Within the past

few months, however, extensive surveys of major metabolic enzymes in natural populations of numerous species of *Drosophila* have been reported¹⁵⁻¹⁷, which allow a meaningful test of the hypothesis outlined here.

Table 1 is a compilation of these studies; the frequencies of polymorphism for as many as fourteen enzymes in individuals collected from a total of ten populations of *Drosophila* (six species) were reported. Although the frequency with which a given polymorphism occurs may vary for the different populations (as the selective factors maintaining it may vary), the examination of data obtained from many populations should minimize the effects of this problem and reveal a consistent pattern, if there are metabolic reasons for certain reactions being sensitive targets of polymorphism-promoting selection. Such a pattern exists, as is clearly demonstrated in Fig. 1, in which the polymorphic allele frequencies of the fourteen enzymes are plotted against their equilibrium constants: the frequencies observed are markedly higher for reactions, with equilibria which lie far to the right.

To obtain an estimate of the average degree of polymorphism associated with each enzyme, the frequency of an enzyme's most common allelic class in each population is multiplied by the sample size of that population, and the products for all populations used to calculate a frequency P

$$P = \sum f_i x_i / \sum x_i$$

where, for sample i , f is the frequency of the most common allele and x is the sample size. P is thus the average frequency, weighted for sample size, with which the class "most common allele" occurs. One may then consider $(1-P)$ as an index of polymorphism, values of which calculated from the reported surveys suggest that a close correspondence exists between the frequency of enzyme polymorphism and the equilibrium constants of the reactions (Fig. 2).

This same correspondence can be seen in the distribution of examined populations in which high frequency polymorphisms ($\geq 4\%$) are reported (Table 1): high frequency polymorphisms are reported in every case where K_{eq} is large, while no high frequency polymorphisms are reported for freely reversible reactions.

Recent data on the frequency of enzyme polymorphism in extensive studies of human populations are consistent with the above findings¹⁸: high frequency polymorphisms are reported for glucose-6-phosphate dehydrogenase and phosphoglucosmutase, and are not found for 6-phosphogluconate dehydrogenase.

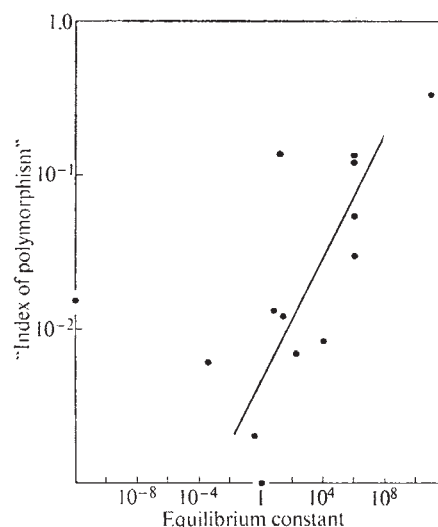


Fig. 2 Relationship of "index of polymorphism" to equilibrium constant. The "index of polymorphism" values are those of Table 1, and reflect the frequency in an average population with which the most common allele of an enzyme does not occur.

Thus, as theoretical considerations predict, a relationship is seen between the occurrence of enzyme polymorphisms and the equilibrium constants of the enzyme reactions involved. The existence of such a relationship argues strongly that enzyme polymorphisms are generally of adaptive significance in nature, and suggests that the magnitude of the observed enzyme polymorph frequencies in natural populations reflects not only the strength of the selection maintaining the polymorphism, but also the "responsiveness" of the metabolic step to that selection.

GEORGE B. JOHNSON

Department of Biological Sciences,
Stanford University,
Stanford, California 94305

Received December 21, 1970; revised March 12, 1971.

- ¹ Lewontin, R., and Hubby, J., *Genetics*, **54**, 595 (1966).
- ² Burns, J., and Johnson, F., *Science*, **156**, 93 (1967).
- ³ Harris, H., *Proc. Roy. Soc., B*, **174**, 1 (1969).
- ⁴ King, J., and Jukes, T., *Science*, **164**, 788 (1969).
- ⁵ Selander, R., and Yang, S., *Genetics*, **63**, 653 (1969).
- ⁶ Kimura, M., *Nature*, **217**, 624 (1968).
- ⁷ Koehn, R., *Science*, **163**, 943 (1969).
- ⁸ Gibson, J., *Nature*, **227**, 959 (1970).
- ⁹ Richmond, R., *Nature*, **225**, 1025 (1970).
- ¹⁰ Simpson, G., *Science*, **146**, 1535 (1964).
- ¹¹ Selander, R., and Yang, S., *Genetics*, **58**, 599 (1967).
- ¹² Selander, R., Hunt, W., and Yang, S., *Evolution*, **23**, 379 (1970).
- ¹³ O'Brien, S., and MacIntyre, R., *Amer. Nat.*, **103**, 97 (1969).
- ¹⁴ Henderson, N., *Ann. N.Y. Acad. Sci.*, **151**, 429 (1968).
- ¹⁵ Kojima, K., Gillespie, J., and Tobari, Y., *Biochem. Genet.*, **4**, 627 (1970).
- ¹⁶ Prakash, S., *Proc. US Nat. Acad. Sci.*, **62**, 778 (1969).
- ¹⁷ Prakash, S., Lewontin, R., and Hubby, J., *Genetics*, **61**, 841 (1969).
- ¹⁸ Harris, H., *Brit. Med. Bull.*, **25**, 5 (1969).
- ¹⁹ Racker, E., *J. Biol. Chem.*, **184**, 313 (1950).
- ²⁰ Johnson, M., in *The Enzymes* (edit. by Boyer P., Lardy H., and Myrback K.), **3**, 407 (Academic Press, New York, 1960).
- ²¹ Morell, D., *Biochem. J.*, **51**, 657 (1952).
- ²² Baranowski, T., *J. Biol. Chem.*, **180**, 535 (1949).
- ²³ Robbins, E., and Boyer, P., *J. Biol. Chem.*, **224**, 121 (1957).
- ²⁴ Harary, I., Korey, S., and Ochoa, S., *J. Biol. Chem.*, **203**, 595 (1953).
- ²⁵ Najjar, V., *The Enzymes*, **6**, 161 (1962).
- ²⁶ Hill, R., and Bradshaw, R., in *Methods in Enzymology* (edit. by Lowenstein J.), **XIII** (Academic Press, New York, 1969).
- ²⁷ Pontremoli, S., Grazi, E., de Flora, A., and Mangiarotti, G., *Arch. Sci. Biol.*, **46**, 83 (1962).
- ²⁸ Kahana, S., Lowry, O., Schulz, D., Passonneau, J., and Crawford, E., *J. Biol. Chem.*, **235**, 2178 (1960).
- ²⁹ Lehninger, A., Sice, J., and Jensen, E., *Biochim. Biophys. Acta*, **17**, 286 (1955).

Evidence for Selective Differences between Cells with an Active Horse X Chromosome and Cells with an Active Donkey X Chromosome in the Female Mule

THE glucose-6-phosphate dehydrogenase (G6PD) locus is known to be X-linked in the horse and donkey^{1,2}. The female mule, an interspecific hybrid of these two species, is an obligatory heterozygote for two electrophoretically distinguishable G6PD alleles^{1,2}.

Studies of the DNA replication patterns of the chromosomes of two female mules by Mukherjee *et al.*^{3,4} have shown that, in about half the cells investigated in each animal, the X chromosome derived from the female parent (the horse X) was late replicating and the X chromosome derived from the male parent (the donkey X), was early replicating; the reverse pattern obtained in the other half of the cells. Hamerton *et al.*⁵ found in three female mules, however, that most lymphocytes and fibroblasts had a late replicating donkey X chromosome and early replicating horse X chromosome. The distribution was

significantly different from the roughly equal representation of cell types noted by Mukherjee *et al.* In the only cell line studied electrophoretically by Hamerton *et al.*, horse G6PD was preferentially expressed, consistent with the hypothesis that the early replicating mammalian X chromosome in females is the active one⁶. The observations of the latter workers suggest that in some female mules, either (1) the donkey X chromosome is preferentially inactivated early in development, or (and) (2) cells with an active horse X chromosome have a selective advantage in at least some tissues during ontogeny.

Table 1 G6PD Phenotypes of Female Mules

Predominant phenotype	Cervical cord	Pancreas	Parotid	Liver	Lung	Thyroid
H > D	20 (18)	19 (12)	23 (15)	9 (6)	8 (8)	8 (6)
H $\approx$ D	1 (1)	4 (4)	4 (3)	12 (7)	11 (5)	12 (9)
D > H	2 (2)	4 (3)	3 (3)	13 (8)	6 (4)	9 (5)

The number of animals with the observed phenotypes are listed. Not all organs from every animal could be investigated, but in twenty-two of the fifty-four animals at least five of the six organs listed in the table were studied. The results for these twenty-two animals are listed in parentheses. The patterns were scored independently by E. B. H. and L. D. B. and the sample rerun if there was a discrepancy or uncertainty (see Fig. 1). Examination of blood samples revealed that the horse band was the usual predominant one here also. Mixing experiments with variable proportions of donkey and horse blood samples with known activity indicated that, when the bands of approximately equal intensity were observed, there was at least 35% activity of the major component in the mixture. In a mule in which expression of donkey and horse phenotype was close to equality, there was no observed difference in the proportion of parental phenotype between reticulocyte-rich and reticulocyte-poor preparations, although there was less activity in the latter. Similarly, there was no observed difference between fresh and stored cells in the proportion of parental phenotype, although activity was lower in stored cells. The specific activities of G6PD in peripheral blood of a young donkey and of a young horse were within 10% of each other. Earlier studies⁸ suggested that the donkey phenotype was preferentially expressed in liver, but this no longer seems likely.

We have investigated this question by studying the G6PD phenotypes of samples from tissues and organs of fifty-four female mules. The results for representative organs are listed in Table 1. Examples of patterns and their interpretation are illustrated in Fig. 1.

There were two organ types: those in which the horse phenotype was usually predominant, and those in which expression was not significantly different from what would be expected on a random basis. In none of the organs studied was the donkey phenotype the usual predominant one. If X inactivation were non-random, and occurred at the early

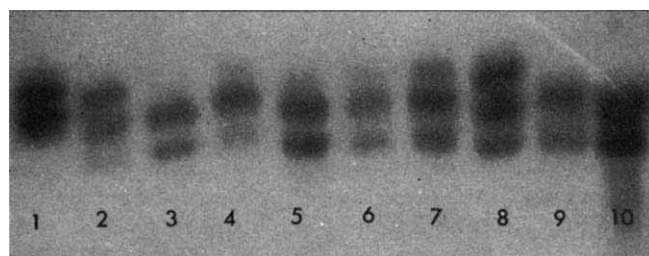


Fig. 1 The bands in the slots at extreme left (1) and right (10) illustrate donkey (D) and horse (H) patterns respectively. The other samples, from pancreas and parotid, were interpreted from left to right as follows: (2) D > H, (3) H > D, (4) ?, (repeat), (5) H > D, (6) H > D, (7) ? H $\approx$ D, (repeat), (8) D > H, (9) H > D. All organs were studied using starch gel electrophoresis in EBT buffer with standard methods⁹ at pH 8.6. Almost all animals were relatively old. Organ samples were collected at the abattoir on dry ice about 60–120 min after death and exsanguination. Blood was obtained in citrate about 5–15 min after death and stored at 4° C.

stage usually hypothesized in the mammalian female embryo, representation of the favoured allele should be relatively uniform in all derived tissues, unless cell selection in particular organs had also occurred. Since *X* inactivation is thought to occur at about the blastocyst stage⁷, before observable organ differentiation, it seems unlikely that preferential inactivation has occurred just in the cell precursors of particular organs. It seems more probable that *X*-linked alleles of the donkey and horse have had different effects during development and maturation, with a relative selective advantage for cells with an active horse *X* chromosome in organs such as the parotid gland. But preferential expression of the horse *X* chromosome in these latter organs did not occur in all animals studied, indicating that the observed phenomenon represents a trend rather than a uniform developmental event in the female mule. Thus discrepancies between studies such as those of Hamerton *et al.* and Mukherjee *et al.* seem likely to reflect sampling differences in animals studied.

An alternative explanation for the observed distribution should be mentioned. Unknown organ specific factors may have differentially suppressed (or enhanced) expression of a particular parental G6PD phenotype (for example, perhaps inhibiting expression of the donkey band in cervical cord, pancreas and so on). Although this seems unlikely, it cannot be completely excluded. But when samples of pancreas with horse phenotype were mixed with samples of other organs which expressed the donkey phenotype, there was no evidence for an inhibiting factor.

ERNEST B. HOOK
LORETTA D. BRUSTMAN

Birth Defects Institute,
New York State Department of Health,
and Department of Pediatrics,
Albany Medical College of Union University,
Albany,
New York 12208

Received February 18, 1971.

- ¹ Trujillo, J. M., Walden, B., O'Neil, P., and Anstall, H. B., *Science*, **148**, 1603 (1965).
- ² Mathai, C. K., Ohno, S., and Beutler, E., *Nature*, **210**, 115 (1966).
- ³ Mukherjee, B. B., Mukherjee, A. B., and Mukherjee, A. B., *Nature*, **228**, 1321 (1970).
- ⁴ Mukherjee, B. B., and Sinha, A. K., *Proc. US Nat. Acad. Sci.*, **51**, 252 (1964).
- ⁵ Hamerton, J. L., Gianelli, F., Collins, F., Hallett, J., Fryer, A., McGuire, V. M., and Short, R. V., *Nature*, **222**, 1277 (1969).
- ⁶ Lyon, M. F., in *Adv. Teratol.*, **1** (edit. by Woolam, D. H. M.) (Logos Press, London, 1966).
- ⁷ Kinsey, J. D., *Genetics*, **55**, 337 (1967).
- ⁸ Hook, E. B., and Brustman, L. D., *Genetics*, **64**, 530 (1970).
- ⁹ Motulsky, A. G., and Yoshida, A., in *Biochemical Methods in Red Cell Genetics* (edit. by Yunis, J. Y.), 51 (Academic Press, New York, 1969).

New Determination of Australopithecine Height

AN estimation of the body height of two robust australopithecines (Sk 82 and Sk 97) is attempted here on the basis of a ratio between femoral head diameter and body height in modern *Homo sapiens*. Coon, using the fibula and tibia from the "Zinjanthropus" living floor at Olduvai, has estimated¹ three different heights for this creature or creatures: 144.5 cm (4 feet 8 inches); 136 cm (4 feet 6 inches); and 152.4 cm (5 feet). Lovejoy and Heiple, using specimens from Sterkfontein (STS 14, STS 34 and TM 1513), have reconstructed² a height for the gracile australopithecines at 106.7 cm–109.2 cm (3 feet 6 inches–3 feet 7 inches). An estimation has never been made, however, for the height of an undisputed robust australopithecine.

Because australopithecines were fully erect and bipedal³ it is possible to apply postcranial information derived from modern *Homo sapiens* to australopithecines; the mechanical relations which hold true for modern bipedal *Homo sapiens* will also apply therefore to bipedal australopithecines. I selected fifty specimens (thirty males and twenty females) at random from the Hamann-Todd Collection of modern *Homo sapiens* in the Cleveland Museum of Natural History. I defined terms and measurements as follows. Femoral head maximum diameter: the diameter of the femoral head taken in the horizontal plane with the femur resting on the condyles and trochanter. Femoral head minimum diameter: the diameter of the femoral head taken at right angles to the plane of maximum diameter. I took care to avoid measuring non-weight bearing surfaces or protrusions. Circumference: the circumference of the femoral head taken at the greatest measurement of weight bearing area on the head. Calculated area: the head of the femur was considered as a half sphere. I used the formula, $\text{area} = 2\pi r^2$ (r , radius), half the value of the surface sphere.

The specimen files of the Hamann-Todd Collection provided body heights. All measurements are in centimetres or, in the case of areas, in centimetres², and were taken with a Vernier calliper accurate to a hundredth of a millimetre and a cloth tape accurate to one millimetre. Rechecking occasional specimens for all measurements showed that the values obtained originally were at least 98% accurate.

Determinations were made to indicate which measurement, area or diameter would relate best with body height. By correlation analysis, I found that the average diameter of the head (midpoint between maximum and minimum) correlated better than the surface area (a correlation of 0.648 as opposed to 0.627) and I used it in all further calculations and considerations.

Using a least mean squares two variable regression I then calculated the line of best fit for the data. The equation of this line is

$$\text{body height} = 13.29 \times + 108.59$$

The average distance of actual body height from this line is 3.2%, or 5.4 cm. I calculated absolute range of error using the body heights furthest removed from the line of best fit even though these heights may be anomalous. This range of absolute error is +8.2%, –7.4%. I then used this formula to predict the body height of Sk 82 as 151 cm (4 feet 11.5 inches) and Sk 97 as 157 cm (5 feet 1.5 inches). Taking average range of error into account, the range of probable heights for these specimens is

Sk 82 146–156 cm

Sk 97 152–161 cm

I see no reason why this method and formula cannot be used to estimate the height of any bipedal, erect hominid within the average range of error.

I thank Miss Patricia Helwig, Assistant Curator of Collections, and the Cleveland Museum of Natural History for their assistance in making the Hamann-Todd Collection available.

PETER E. BURNS

Department of Anthropology,
Case Western Reserve University,
Cleveland, Ohio 44106

Received April 6, 1971.

- ¹ Coon, C. S., *The Origin of Races*, 285, 294 (Knopf, New York, 1969).
- ² Lovejoy, C. O., and Heiple, K. G., *Amer. J. Phys. Anthrop.*, **32**, 33 (1970).
- ³ LeGros Clark, W. E., *The Fossil Evidence for Human Evolution*, 157 (University of Chicago Press, Chicago, 1964).

BOOK REVIEWS

Empiricist Physician

Fundamenta Medicinae. By Friedrich Hoffmann. Translated and introduced by Lester S. King. (History of Science Library: Primary Sources.) Pp. xxvi + 142. (Macdonald: London; Elsevier: New York, January 1971.) £3.

THE seventeenth century was fertile with new ideas in medicine and science. Long established Aristotelian doctrines began to crumble before the assault of Baconian empiricism and atomism modified by Descartes, Gassendi and Boyle: the emergence of the iatrochemical and iatromechanical "schools" of medicine completed the rout. It is tempting to classify seventeenth century physicians into various "schools" such as Galenist, iatrochemist or iatrophysicist, but these categories, favoured by latter-day interpreters, were not so clearly defined. In spite of the increased knowledge of scientific medicine, adherents of the chemical or mechanical schools did not really succeed in discarding Galenic remedies or dispensing with the basic tenets of humoral pathology. In this respect Friedrich Hoffmann (1660–1742) was typical of his times.

Born in Halle, Hoffmann studied medicine at Jena and Erfurt, qualifying in 1681. After visiting medical schools in France, Holland and England (where he met Boyle) he returned to practise in Minden and Halberstadt. In 1693 Frederick III invited him to become Professor of Medicine at Halle and, apart from a few years in Berlin as court physician, Hoffmann spent the rest of his life there as a university professor. His interest in medical education led him in 1695 to publish his *Fundamenta Medicinae* as an introduction to systematic medicine.

Hoffmann's faith in scientific medicine is clear from his introduction. "Mechanics alone, wedded to anatomy and chemistry," he wrote, "has so far led natural and medical science from a low position to an exalted one." Although he was able to interpret physiological processes in mechanical terms (his theory of disease too is a mixture of humoral pathology and atomic theory). Hoffmann found it difficult to explain mental processes in similar terms. After removing the theological concept of "soul" beyond

the realm of his treatise, Hoffmann tried to reconcile the rational, volitional functions of the mind with the mechanical functions of the body. He postulated that sensory perception and muscular movement were due to the animal spirits controlled by the sensitive soul—a concept expounded by Willis in *De Anima Brutorum* (1672). His diagnostics, hygiene and therapeutics were concerned with the "art" of medicine and, like Sydenham, Hoffmann followed Hippocratic principles based on generalizations drawn from his own experience. He regarded body fluids as the site of disease and stressed the diagnostic importance of analysing blood, saliva, perspiration, lymph, urine and "spirits". An example of his diagnostic empiricism is the statement that "red urine without fever indicates scurvy". The maintenance of health he thought depended on the balanced interaction of the "six non-naturals—air, food and drink, sleep, useful activities and rest, evacuation and repletion and the emotions and passions". Hoffmann's therapeutics were based on his own experience of drug action within the theoretical framework of humoral pathology. He used Galenicals rather than chemical remedies, and although his theory of drug action was essentially mechanical, his actual prescribing was reassuringly empirical. Thus there was a conflict between the art of the physician using drugs based on past experience, and the scientific doctor anxious to propagate new discoveries in physics and chemistry.

This small volume precisely translated and usefully introduced by Dr Lester King is of general interest to the medical historian and essential reading for the seventeenth century specialist.

KENNETH DEWHURST

Small Mammals

Energy Flow Through Small Mammal Populations. Edited by K. Petrusewicz and L. Pyszkowski. (Proceedings of IBP Meeting on Secondary Productivity in Small Mammal Populations, held in Oxford, England, July 29th to August 2nd, 1968.) Pp. 298. (Polish Scientific: Warszawa, 1969/1970.)

THIS volume contains the proceedings of a meeting held under the auspices

of the International Biological Programme and yet, from the contents, one might get the impression that work on small mammals is non-existent in Africa, Asia, Australasia and South America. Of the fifty-six participants from fourteen countries, twenty-two came from central or eastern Europe five from North America and the remainder from western Europe. Twenty-two of the thirty-one contributions were written by ecologists from eastern Europe and surely this emphasizes the activity and leadership of these scientists within the context of IBP small mammal ecology.

The contents refer primarily to European work but this does not detract from their importance to small mammal ecology throughout the world. The contributions are grouped into four major sections: (1) standing crop evaluations and dynamics of numbers; (2) food resources and bioenergetic parameters; (3) reproduction and survival and (4) energy flow and productivity estimations. The eleven papers in the first section critically examine methods for estimating population density. It is clear that none of the currently used methods are entirely satisfactory for making absolute density estimates; however, both the mark-release-recapture and the standard minimum removal methods can, when combined with a knowledge of home range and residency, give rise to acceptable estimates. Ingenious methods involving the use of coloured baits and/or radioisotopes are suggested for the determination, among other things, of residency and home range.

The second section comprises nine papers and these, although dealing with the common theme of food resources and bioenergetic parameters, are a miscellaneous collection. In one instance the effect of small mammals on vegetation is considered, in others the effect of different food types on the animals is discussed. Yet other papers are methodological in nature, or deal with seasonal changes in the energy content or respiratory rate of single species.

Two of the papers in the third section tackle the difficult problems of estimating the number of young born into a population and the average

length of life of small mammals. Criticism is levelled at the practice of assuming exponential population growth in certain species and different methods of estimating longevity are compared.

The final section on energy flow and productivity estimations contains six papers. It is interesting to note that although a bank vole has an average life expectancy of up to 3.66 months it is clear that some 60 per cent of its production occurs within the first 0.75 months of its life. This underlines the necessity of finding ways to study what is normally regarded as a non-trappable part of the population. The same conclusion is reached in another paper which deals with the field vole. The remaining papers are primarily concerned with food consumption by small rodents and it would appear that these animals rarely eat more than 1 to 2 per cent of the primary production potentially available to them. This suggests that food might not be a limiting factor to the size of some populations, although one author is careful to point out that food might become a critical factor which limits those overwintering populations which follow a peak in vole numbers.

Clearly, this volume summarizes a wealth of information about diverse matters relating to energy flow through small mammal populations. Typographical errors occur frequently in the text but in no instance did I find them particularly misleading and therefore I have refrained from detailing them. In conclusion it must be said that any mammalogist or ecologist professing an interest in small mammal population density estimates, production or energy flow would be doing himself a disservice if he did not consult this publication.

JOHN PHILLIPSON

havioural science, especially in its scientific and research aspects, and, hopefully, to "constitute a basis for an informed, effective national policy to strengthen and develop" these fields. With the public view of psychiatry dominated by the images of the couch and the asylum, the treatment of neurotic private patients by psychoanalysts, and the confinement of psychotic patients in large mental hospitals, there is certainly room for an entertaining and stimulating review of the areas of expansion in modern psychiatry.

Although this volume provides a fairly wide ranging survey of the field of recent and current research in psychiatry, the survey is patchy and by no means as up to date as one expects from a work published in 1970—much of it could have been written five years ago or more. Psychoanalysis is perhaps over-represented for a study of behavioural science; and the emphasis is almost entirely on American work. The presentation is terse and irregular, and manages to be too specific and technical for the lay reader in many passages, as well as too vague and lacking in detail for the professional reader. There is an intelligent and reasonably selective list of annotated references.

The style of writing is turgid and uniformly woolly, which is unpardonable when dealing with such a lively and potentially exciting subject. The layout and design are singularly uninspiring, and the long slabs of grey type are not always easy to read. Perhaps the major weakness of the book is the common one of aiming at a great many targets and missing most of them. It is neither sufficiently clear and attractive to interest and inform many members of the informed public, nor sufficiently critical and specific to be really helpful in guiding future research and developments in psychiatry.

MICHAEL A. SIMPSON

background of non-critical optical transitions from the smaller contribution due to the critical points, which are nevertheless essential in the adjustment of theoretical band structures.

The contributions by Button and Kaplan on spectroscopy at sub-millimetre and far infrared wavelengths, respectively, bear on semiconductor problems. The use of high frequency photons to excite cyclotron resonance or some other transition between allowed electron states makes possible an order of magnitude increase of resolution compared with that available at microwave frequencies and allows the application of Fourier spectroscopy and the use of resonant photoconductivity as a detection system.

The optical properties of the alkali halides and the inert gases seem to be the simplest possible, and several papers in these conference proceedings concentrate on these materials. Optical properties are evidently better understood than for semiconductors. A paper by Zawadzki develops the analogy between free relativistic electrons and electrons in a narrow gap semiconductor, applying the ideas to the behaviour of electrons in crossed electric and magnetic fields and especially to the optical properties of semiconductors under such conditions. Moriya's review of optical excitation of magnons in spin systems describes a field of growing interest rather sketchily and with no experimental details, only references to the original literature.

Such papers draw attention to the conflict between the more conventional review literature and conference proceedings, in that the inadequacies of some of the reviews in this publication force the reader back to the original literature at an earlier stage than is usual in the study of a review. If the excessive cost of this book is set beside its value to the physicist, it is probably not a book to own but one to be borrowed from a library.

ALEXANDER D. C. GRASSIE

Scientific Psychiatry

Psychiatry as a Behavioral Science. Edited by David A. Hamburg. (The Behavioral and Social Sciences Survey: Psychiatry Panel.) Pp. xii+114. (Prentice-Hall: Englewood Cliffs, New Jersey, 1970.) \$5.95.

SOMEONE once described a camel as a horse that had been designed by a committee. A book written by a committee starts off at a similar disadvantage. This work, one of a series of reports for the public initiated by the US National Academy of Science and the Social Science Research Council, was prepared by a distinguished panel of American scientists. It aims to provide a comprehensive review of psychiatry as a be-

Poor Substitute

Optical Properties of Solids. Edited by E. D. Haidemenakis. (Lectures and Seminars presented at the Fifth Chania International Conference, held at Chania, Crete, Greece, June 30–July 10, 1969.) Pp. xv+520. (Gordon and Breach: New York and London, November 1970.) £12.25; \$9.50.

THIS publication covers optical studies in the alkali halides, the inert gases, semiconductors and magnetic insulators but gives only slight coverage to optical studies of metals. Of especial interest is the review by Seraphin of the use of modulated reflectance in the study of band structures; he shows how the technique can be used to separate the massive

Analysing Proteins

Protein Sequence Determination: Methods and Techniques. By S. Blackburn. Pp. ix+292. (Marcel Dekker: New York, September 1970.) £8; \$16.75.

THIS excellent account of protein sequence analysis will be useful to chemists, biochemists, molecular biologists and others who require a practical guide to the subject. Automated analysis is still very much in its infancy and, as the author points out, sequencing a protein is not yet a routine operation, each protein presenting a problem in its own right. In twenty chapters the sequencing strategy is revealed with the enthusiasm to be expected from an experienced worker in the field.

The book covers cleavage of disulphide linkages; specific and non-specific cleavage of proteins by enzymes, electrophilic reagents, acid and alkali; fractionation of mixtures of peptides by precipitation, gel filtration, ion exchange chromatography, paper chromatography and electrophoresis; end group determination, ordering the peptide fragments, radioactive techniques; and finally, mass spectrometric and X-ray crystallographic techniques. The latter techniques have for good reasons been discussed only briefly, but sufficient information is provided to make possible a useful comparison of data obtained by X-ray analysis with those obtained by conventional chemical techniques. At the beginning of each chapter the principles involved are stated concisely in a homely style and these are followed by practical details of experimental procedures. An excellent job has been done in selecting these practical examples, most of them allowing the worker to take his problem directly to the bench without reference to the original literature. A list of sources is, however, available at the end of each section.

The book is not overburdened with tables and figures, and abounds in sound advice and hints on how to avoid pitfalls. It forms a companion volume to the author's earlier *Amino Acid Determination: Methods and Techniques*, but can be read and used independently. The level of proof reading was quite good although a curious chapter heading was missed on page VIII. The publisher could have been a little more generous with the subject index. Overall the book fulfils its aims and should be well received by research workers who have an interest in peptide and protein sequence data.

J. LEGGETT BAILEY

Chain Genetics

The Genetic Markers of Human Immunoglobulins. By R. Grubb. (Molecular Biology, Biochemistry and Biophysics, Vol. 8.) Pp. xii+152. (Chapman and Hall: London; Springer-Verlag: Berlin and New York, November 1970.) £4.90.

THE immunoglobulins (Igs) are unique among proteins in their diversity of structure and function. The discovery in 1956 of genetic markers on human and rabbit Igs by the author of this book and by Oudin, respectively, was therefore of particular significance. The subsequent intensive study of these markers has provided an essential framework for understanding the genetic control of antibody synthesis, and has delineated several features of great interest with regard to the assembly of antibody molecules and

the differentiation and cooperation of cells which produce antibodies. Genetic markers have also been useful in studies of the phylogeny, ethnic features and ontogeny of immunoglobulins, and forensic pathologists have used them with practical effect in cases of disputed paternity and confusion of newborn infants in maternity hospitals. They can be of unique value in the latter case because the infant's genotype for Ig synthesis is not expressed at birth and its circulating IgG is for practical purposes exclusively of maternal origin.

Genetic markers are now known for three of the seven major classes of immunoglobulin heavy and light chains. Identification of their many polymorphic forms involves the use of human antisera which specifically agglutinate red blood cells coated with human immunoglobulin. The anti-human γ -globulins used in these tests originate principally in patients who have had multiple blood transfusions or in subjects who may be apparently healthy or afflicted with rheumatoid arthritis. The clinical significance of these antibodies in the latter context and their possible role in the pathogenesis of disease are matters of continuing controversy and interest.

All these intriguing ramifications of an important topic are lucidly outlined in the first eighty-five pages of this admirable, small book. The text is supported by a wealth of tabulated data and a concluding appendix of thirty pages provides a comprehensive source for the original references, mode of detection, ethnic distribution, molecular localization and inheritance of some thirty genetic factors which have been detected on human immunoglobulins. This book will be of great value as a succinct and authoritative source for immunologists, geneticists, clinicians and others interested in the many sided problems of antibody synthesis. It is regrettable that the author's economy and accuracy were not emulated by the publishers who are responsible for the inflated price and several printing errors—some diverting, such as that on page 82, which concerns "the mouse lamb dalight chains" of IgM. S. COHEN

Vibrations

Low-Frequency Vibrations of Inorganic and Coordination Compounds. By John R. Ferraro. Pp. xiv+309. (Plenum: New York, 1971.)

IMPORTANT developments in far infrared instrumentation during the past ten years have led to a considerable increase in the published data on low frequency vibrations of molecules and ions, which are not only of interest to spectro-

scopists but of potential value to other chemists. In this book the author, who has published a considerable number of research papers in this field, sets out to demonstrate the usefulness to inorganic chemists of infrared absorption measurements below 650 cm^{-1} on inorganic compounds.

A brief introduction sketches the history of far infrared measurements and the experimental and interpretative problems of this region of the spectrum, and is followed by a chapter describing commercial instruments including interferometers, some in considerable detail but others only in briefest outline. A comparison of the relative merits of spectrometers and interferometers, probably of greater general interest than the rest of the chapter, is unfortunately presented in brief tabular form, with references to other texts. The third chapter summarizes useful data on window materials and some common organic solvents. Calibration is also briefly discussed but one is referred to other texts for many of the calibration data. The fourth chapter sketches the use of inelastic neutron scattering and high pressure techniques in vibrational studies, again with references to other sources for details.

The next four chapters, comprising more than two-thirds of the book, present data (including those from Raman measurements) and discussion on metal-oxygen, metal-halogen, metal-nitrogen and miscellaneous metal-ligand vibrations. Many of the data are in tabular form and the presentation is similar to that in other books of this type. The final chapter very briefly summarizes some results on ion-pair and lattice vibrations, clathrate vibrations and low frequency electronic transitions. Two appendices give tables of crystal site symmetries and subgroup correlations, and describe a method for factor group analysis of crystal vibrations.

This book undertakes to cover a very wide field embracing almost the whole of metal-ligand vibrations, which is scarcely reduced by the rather artificial limitation to frequencies below 650 cm^{-1} . In order to save space much use is made of references to other texts for detailed discussion, and on many topics no detailed or critical account is given. References are included up to 1969, but, the author says, the book does not claim complete coverage of topics or references, and there are some obvious gaps. The book therefore seems to be chiefly of value to newcomers and non-specialists in this field, giving in one volume brief accounts of both experimental and interpretative matters. While also of use to specialists as a recent source of references, its value as a convenient source of data is somewhat reduced by its lack of comprehensiveness on details.

J. A. CREIGHTON

Spectra Galore

Spectroscopy in Inorganic Chemistry. Vol. 1. Edited by John R. Ferraro and C. N. R. Rao. Pp. xiii + 410. (Academic: New York and London, October 1970.) \$19.50; £9.10.

THE two volume treatise of which this volume is the first part is more specialized than its title suggests. It describes recent advances in inorganic spectroscopy but omits well known aspects of spectroscopy such as electronic or vibrational spectroscopy and their applications to coordination chemistry as well as lengthy discussions of instruments and methods. The articles are intended not as complete compilations but as reports of the present position and of future potentialities.

There has not been much time for some of the new results to be digested, and many of the chapters are oriented towards methods and methodology (even when these can be found elsewhere) rather than towards the interpretation of spectra in terms of structure and bonding. Many chapters are anecdotal rather than correlative, some are too elementary. Thus section D of the chapter on high-resolution NMR begins: "The electronic environment in molecules does one more subtle thing. It couples magnetic nuclei present in the same molecule. Such a coupling is experimentally observed as a splitting of NMR lines." (Surprise, surprise.) This chapter describes some useful inorganic studies, but fails to mention superconducting magnets, liquid crystals and Fourier transforms.

Some of the chapters are competently written, a few well written. The foreign language literature, however, and its English translations, is often poorly served. The first article refers to one German and to two (translated) Japanese papers in 65 references. The review of Mössbauer spectroscopy, which is a German invention, refers to two German papers (by Mössbauer), two Japanese and one (translated) Russian paper among 90 references. Editors (on both sides of language barriers) should encourage their authors in scientific internationalism, for this lack of effort, which is not necessarily chauvinism, is only too common. In a volume costing £9 (£1 per article of about 45 pp.) we expect coverage of the less accessible, and no less relevant, parts of the chosen fields.

There are other examples of parochialism. Thus the description of the early work in matrix isolation spectroscopy refers to Pimentel but not to Norman and Porter, Rice or Lewis and Lipkin¹.

While counting references, we could congratulate the authors of the chapter on soft X-ray spectroscopy on their activity in this field, for 23.5% of their references are to their own work. They tell us that their examples were chosen to show "typical results", but the references, if not the text, should aim at a reasonable

coverage and should mention other useful accounts that are available². Their suggestion (p. 210) that the future significance of this method lies in the interpretation of spectra rather than in the determination of concentration is misleading; they themselves report (p. 242) that the analysis of rocks by X-ray emission spectroscopy was shown (1965) to be better than with wet chemical techniques.

As the book goes on, the proof reading flags, and a strange chemistry, of nitrogen, cyanides and ferrocyanides, develops. We need these reviews; but the high cost of distribution justifies a plea for a high standard of preparation.

J. MASON

¹ Norman, I., and Porter, G., *Nature*, 174, 508 (1954); Rice, F. O., and Freamo, M., *J. Amer. Chem. Soc.*, 73, 5529 (1951); 75, 548 (1953); Lewis, G. N., and Lipkin, D., *J. Amer. Chem. Soc.*, 64, 2801 (1942).

² *Practical X-ray Spectrometry* by R. Jenkins and J. L. De Vries, Macmillan, London; Springer, New York (1971); and "X-ray Fluorescence Spectrography" by K. Norrish and B. Chappell, in *Physical Methods in Determinative Mineralogy*, Academic Press, London (1967).

Hot Polymers

Thermal Stability of Polymers. Vol. 1. By Robert T. Conley. (Monographs in Macromolecular Chemistry.) Pp. x + 644. (Marcel Dekker: New York, October 1970.) \$44.50.

THE wide uses to which polymeric materials, especially those of synthetic origin, are now applied make it imperative that information on properties and performance should be as complete as possible. Plastics, rubbers and fibres are often subjected to environmental conditions which may have a profound effect on their chemical, physical and mechanical properties. Behaviour at elevated temperatures is an important factor when selecting suitable materials, and this book provides a welcome collective survey on thermal and thermal/oxidative degradation. Edited by Professor Conley of Wright State University, Dayton, Ohio, it has fourteen chapters, by experts in US academic, industrial and research organizations and one contributor from the UK (Dr Wright of the Royal Aircraft Establishment, Farnborough).

In two initial chapters, Professor Conley discusses the general concepts of polymer properties, degradation processes, molecular structure, and stability criteria, drawing attention to structures which contribute to thermal resistance. Chapters by R. H. Boyd and A. V. Tobolsky and co-authors then review the more theoretical aspects of thermal depolymerization, scission processes and polymer oxidation, providing a background essential for appreciation of later chapters where

important classes of polymers are considered. These deal with polyolefines (R. H. Hansen), rubbers (E. M. Bevilacqua) and fluorocarbon polymers (W. W. Wright). Professor Conley and co-authors provide chapters on vinyl and acrylic polymers; on polyamide, polyimide and related condensates, polyesters and polyethers; on thermosetting polymers (phenolic, nitrogen, furan and epoxy resins); and on cellulosic products. The remaining chapters are by G. P. Shulman who deals with heterocyclic polymers and to some extent covers the same field as Conley's chapter ten, and by J. Economy and J. H. Mason on the behaviour of some inorganic polymers.

Each chapter is accompanied by lists of references to the more important papers (usually up to 1967), so giving an extensive but sometimes non-critical review since the treatment varies with the individual contributors. While the editor has endeavoured to bring the contributions together in a uniform manner, some repetition or overlap is evident (particularly in the tenth and thirteenth chapters). To some extent this may be an advantage in that the chapters become self-contained. There are places where the reader will see omissions or be critical of the authors' interpretation of published information, evident in discussions on acrylic, vinyl and olefine polymers. Another feature is the apparent insufficient attention given to technological implications, particularly on the use of stabilizers, antioxidants and modified process conditions, all of major importance in polymer-using industries. Again, one notes that while there is much on the degradation of polyacrylonitrile, the bearing of this on carbon fibre formation is not stressed; nor are polyurethanes dealt with.

Author and subject indexes are provided, although the latter could have been more comprehensive, and I noticed some typographical errors throughout the text.

The book presents a useful addition to the literature and those who are concerned with the behaviour of polymers in conditions where thermal and oxidative influences are encountered will find it to be of particular value. Unfortunately, the high price may well limit its general availability and it is likely to be consulted in libraries rather than purchased for personal use. I look forward to the second volume, now in preparation, and although the possible content has not been indicated it is hoped that this will be complementary to the first volume and perhaps be concerned with other polymer types and with thermal degradation as it affects processing and properties rather than chemical structure.

R. J. W. REYNOLDS

CORRESPONDENCE

Smoking and Cancer

SIR,—In his letter (*Nature*, 231, 543; 1971) on smoking and lung cancer, Dr Sterling states that he had set up a panel to provide authoritative guidance on the link between smoking and disease, and invited Dr Cuyler Hammond to meet this panel. He also requested Dr Hammond to provide the panel with the unpublished data from his own studies: Dr Hammond refused.

He then develops a highly personal attack on Dr Hammond in which he implies that this refusal casts serious doubt on Dr Hammond's reports on the association between smoking and lung cancer.

More seriously, however, Dr Sterling's letter also implies that this refusal by Dr Hammond casts similar doubt on other studies on the relationship between lung cancer and smoking. Thus, Dr Sterling impugnes the integrity and competence of the numerous scientists and committees in many countries who have reviewed exhaustively the extensive data published on this subject^{1,2}. If Dr Sterling disagrees with their conclusions he should do so in a carefully documented critique of all the published data rather than by singling out Dr Hammond's study for personal criticism.

I suspect the reason for Dr Hammond's unwillingness to meet with Dr Sterling's panel is that he does not feel that his time would be best spent discussing and presenting data to yet another group of individuals apparently unfamiliar with the material in the widely explored area of cigarettes and lung cancer. There must be few experts in this field who have studied the literature and not yet reached a decision.

Thus, I believe that Dr Hammond's colleagues will consider that Dr Sterling's tasteless attack constitutes a form of blackmail, and that he was fully justified in his decision not to discuss his data with Dr Sterling's panel. Further, many will draw the conclusion that this attack implies that any scientist who fails to make his data available to any Tom, Dick or Harry is lacking in credibility. This attempt to discredit scientific data and conclusions by character assassination through inference should not be part of the modern scientific approach, and I am surprised that this letter was published in *Nature*.

I have disagreed from time to time with the American Cancer Society on its

comments on individual scientific papers, but I have not doubted its good faith and the basic correctness of its wish to bring to public notice the danger of a popular habit, which at best is a thankless task.

Lastly, Dr Sterling should confirm that neither he, nor his department nor his research, are supported by the grant reported to have been made recently to his university by certain industrial interests.

Yours faithfully,

JOHN HIGGINSON

*World Health Organization,
International Agency for Research on
Cancer,
16 Avenue Maréchal Foch,
69 Lyon (6ème)*

¹ *Smoking and Health Report of the Advisory Committee to the Surgeon General of the Public Health Service* (Public Health Service Publication No. 1103, 1964).

² *Smoking and Health Now* (A Report of the Royal College of Physicians, 1971).

Defoliation by Gypsy Moth

SIR,—In the article "St George versus a Gypsy Moth" (*Nature*, 231, 142; 1971), *Nature* shows signs of adopting the style of its American contemporary, a consummation devoutly to be abhorred. Your Washington correspondent says that the battle of words over the gypsy moth "has been a walk-over for those who argue that gypsy moths may in all likelihood be less pernicious than the measures taken to exterminate them". Who decided this? On June 24, 1970, the *New York Times*, which is scarcely a friend of pesticides, reported from Riverhead, Long Island (p. 59): "... The caterpillars arrived in large cans and plastic bags brought to the legislative session by more than 50 irate residents of the communities of Shirley, Mastic and Ridge. The residents demanded immediate emergency action to curb this year's invasion which is, according to some agricultural experts, the largest since the moth was introduced into this country in 1869. 'We are in a state of emergency', Mrs Helen Gaylord, of 32 Maple Avenue, in Shirley, told the legislature, 'Our children cannot go out. Our pools are finished for the summer. It's a question of survival—the caterpillars or us.'"

Nature states that the USDA, "like many bureaucracies, is kept in motion by force not of reason but of habit", a

generalization that also could be unkindly applied to Washington correspondents. Your correspondent also asks the rhetorical question: "Why does the Department of Agriculture persist in pursuing a programme of which the objectives are ill defined, the benefits clearly temporary, the biological consequences unknown because of inadequate research, and the intellectual foundations so shoddy as to destroy any pretensions to academic respectability the department might still hold?"

The objective of the programme is clearly defined—kill gypsy moth caterpillars. All benefits can be described as temporary, and all biological consequences as unknown. The final phrase is merely name-calling. Stripped of its emotional rhetoric, the article in *Nature* is a one-sided plea for not interfering with "the natural evolution which must take place while gypsy moths find their natural niche" (column 3). This is the standard neo-Luddite plea against pest control. It is voiced most commonly by dwellers in air-conditioned urban buildings. Meanwhile, after a century to "find their natural niche", the gypsy moths are on the move, and are defoliating vast new areas of forest and woodland¹.

Yours faithfully,

THOMAS H. JUKES

*Berkeley,
California*

¹ Nichols, J. O., *Pennsylvania Forests, Fall 1970* (Pennsylvania Forests Association, 5221 E. Simpson Street, Mechanicsburg, Pennsylvania).

Reports and Publications

not included in the monthly Books Supplement

Great Britain and Ireland

Forestry Commission. Booklet No. 28: Gwydyr Forest in Snowdonia—a History. By Donald L. Shaw. Pp. vii+107+26 plates. 40p net. Bulletin No. 42: Conifer Woolly Aphids (Adelgidae) in Britain. By C. I. Carter. Pp. iv+51. 75p net. Forest Record No. 74: Development of Glasshouse Techniques for Early Progeny Test Procedures in Forest Tree Breeding. By R. B. Herbert. Pp. 34. 20p net. Forest Record No. 75: Design, Construction and Maintenance of Earth Dams and Excavated Ponds. By E. F. Granfield. Pp. 75. 17p net. Booklet No. 30: Metric Conversion Tables for Forestry. Compiled by J. E. Everard. Pp. v+71. 50p net. (London: HMSO, 1971.) [245] Queen Mary College, University of London. Annual Report 1969/1970. Pp. 149. (London: Queen Mary College, 1971.) [255] Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 269. No. 1199 (13 May 1971): A Discussion on Architectural Aerodynamics. Organized by M. J. Lighthill and A. Silverleaf. Pp. 321–554+plates 8–12. £6.50; \$16. Vol. 269. No. 1200 (13 May 1971): Splashing of Water Drops on Solid and Wetted Surfaces: Hydrodynamics and Charge Separation. By Z. Levin and P. V. Hobbs. Pp. 555–586+plates 13–16. £1.10; \$2.85. (London: Royal Society of London, 1971.) [255]

Other Countries

Australia: Commonwealth Scientific and Industrial Research Organization. Annual Report of the Division of Meteorological Physics, 1969/1970. Pp. 41. Division of Forest Products—Research Review 1969/1970. Pp. 55. Rangelands Research Unit—Report 1968/1969, and 1969/1970. Pp. 49. (Melbourne: CSIRO, 1971.) [304]

Zentralblatt für Pharmazie, Pharmakotherapie und Laboratoriumsdiagnostik, 6. Band 109, 1970. (Berlin: Veb Verlag Volk und Gesundheit, 1970.) [304]

Queen Victoria Museum and Art Gallery, Launceston, Tasmania. Annual Report 1968/1969. Pp. 8+4 plates. Records of the Queen Victoria Museum. No. 35: *Allanaspides helonomus* Gen. Et. Sp. Nov. (Crustacea: Sycarida) from Tasmania. By R. Swain, I. S. Wilson, J. L. Hickman and J. E. Ong. Pp. 7. No. 36: The Geology and Petrology of the Tertiary Volcanic Rocks of the Tamar Trough, Northern Tasmania. By F. L. Sutherland. Pp. 58. (Launceston: Queen Victoria Museum and Art Gallery, 1970 and 1971.) [304]

Australia: CSIRO. Division of Fisheries and Oceanography Technical Paper No. 30: The Hydrological Environment of Moreton Bay, Queensland, 1967-68. By B. S. Newell. Pp. 35. (Melbourne: CSIRO, 1971.) [304]

The Royal Society of London (1660-1970). By Sir David Martin. (Lecture delivered to the Israel Academy of Sciences and Humanities on May 7, 1970.) Pp. 15. (Jerusalem: The Israel Academy of Sciences and Humanities, 1970.) [304]

Journal of Vocational Behavior, Vol. 1, No. 1, January 1971. Edited by Samuel H. Osipow. Pp. 1-204. Vol. 1, 1971 (4 issues). Institutional subscriptions: \$25. Private subscriptions (for the subscriber's personal use only): \$10. (New York: Academic Press, Inc., 111 Fifth Avenue, 1971.) [304]

Commonwealth of Australia. Department of Supply. Australian Defence Scientific Service—Defence Standards Laboratories. Technical Memorandum No. 34: Comparison of Tentacle Extracts from *Chirospalmus quadrigatus* and *Chironex fleckeri*. By T. E. B. Keen. Pp. 8. Report 377: Further Studies on the Biochemistry of the Toxins from the Sea Wasp *Chironex fleckeri*. By H. D. Crone and T. E. B. Keen. Pp. 8. (Ascot Vale, Victoria: Chief Superintendent, Defence Standards Laboratories, 1970.) [304]

US Department of the Interior: Fish and Wildlife Service. Bureau of Sport Fisheries and Wildlife. Resource Publication No. 87: Review of the Problem of Birds Contaminated by Oil and Their Rehabilitation. By John W. Aldrich. Pp. iv+23. \$0.35. Resource Publication No. 65: Effects of Surface Mining on Fish and Wildlife in Appalachia. By Joseph A. Boccardy and Willard M. Spaulding, Jr. Pp. iv+20. \$0.35. Resource Publication No. 68: Effects of Surface Mining on the Fish and Wildlife Resources of the United States. By Willard M. Spaulding, Jr., and Ronald D. Ogden. Pp. iv+51. \$0.60. (Washington, DC: Government Printing Office, 1970 and 1971.) [35]

Carnegie Institution. Annual Report of the Director of the Department of Terrestrial Magnetism, 1969/1970. (Reprinted from *Carnegie Institution Year Book* 69 for the year July 1, 1969—June 30, 1970.) Pp. 367-550+7 plates. (Washington, DC: Carnegie Institution, Department of Terrestrial Magnetism, 1971.) [35]

World Health Organization. Technical Report Series, No. 464: Joint FAO/WHO Expert Committee on Brucellosis—Fifth Report, Geneva, June 29-July 6, 1970. Pp. 76. (Geneva: WHO; London: HMSO, 1971.) 4 Sw. francs; 40p; \$1.25. [35]

Official Records of the World Health Organization, No. 188: The Work of WHO 1970—Annual Report of the Director-General to the World Health Assembly and to the United Nations. Pp. xvii+305. (Geneva: WHO; London: HMSO, 1971.) 9 Sw. francs; 90p; \$3. [35]

National Institute of Nutrition, Hyderabad. Annual Report for the period October 1, 1969, to September 30, 1970. Pp. viii+140. (Hyderabad: National Institute of Nutrition, Indian Council of Medical Research, 1970.) [35]

Population Bulletin, Vol. 27, No. 1 (February 1971): The Future Population of the United States. Pp. 32.

(Washington, DC: Population Reference Bureau Inc., 1971.) [45]

Bulletin of the Museum of Comparative Zoology, Harvard University. Vol. 141, No. 1 (December 17, 1970): West Indian Xenodontine Colubrid Snakes—Their Probable Origin, Phylogeny, and Zoogeography. By Vincent J. Maglio. Pp. 53. (Cambridge, Mass: Museum of Comparative Zoology, Harvard University, 1970.) [45]

Fondation Universitaire. 50e Rapport Annuel, 1969/1970. Pp. 161. (Bruxelles: Fondation Universitaire, 1971.) [45]

US Department of the Interior: Geological Survey. Bulletin 1315: Geochemical and Geological Reconnaissance in the Seventymile River Area, Alaska. By Sandra H. B. Clark and Helen Foster. Pp. iii+21. \$0.25. Bulletin 1313-A: Variable Azimuth Schlumberger Resistivity Sounding and Profiling Near a Vertical Contact. By Adel A. R. Zohdy. Pp. iv+22+1 plate. \$0.65. Bulletin 1333: Geology of the McCarthy B-4 Quadrangle, Alaska. By E. M. MacKevett, Jr. Pp. iii+31. \$0.25. (Washington, DC: Government Printing Office, 1970 and 1971.) [45]

Umweltforschung—Aufgaben und Aktivitäten der DFG 1950 bis 1970. Pp. 184. (Bonn-Bad Godesberg: Deutsche Forschungsgemeinschaft, 1971.) [45]

Alfred P. Sloan Foundation—Report for 1970. Pp. viii+72. (New York: Alfred P. Sloan Foundation, 1971.) [65]

Science Council of Canada. Report No. 11: A Canadian STOL Air Transport System—a Major Program. Pp. 33. \$0.75. Report No. 12: Two Blades of Grass—The Challenge Facing Agriculture. Pp. 61. \$0.75. Background Study for Science Council of Canada. Special Study No. 20: Prospects for Scientists and Engineers in Canada. By Frank Kelly. Pp. 61. \$1. (Ottawa: Information Canada, 1971.) [65]

Smithsonian Contributions to Zoology. No. 82: Pontonilid Shrimps from the Ninth Cruise of R/V *Anton Bruun*, IIOE, 1964. 1: *Palaemonella* Dana and *Periclimenes* Costa. By A. J. Bruce. Pp. 13. \$0.25. No. 83: The Distribution and Patterns of the Major Arteries of the Iguanids and Comments on the Inter-generic Relationships of Iguanids (Reptilia: Lacertilia). By George R. Zug. Pp. 23. \$0.40. No. 72: Revision of the Fish Genus *Ecsenius* (Blenniidae, Blenniinae, Salarini). By Victor G. Springer. Pp. 74. \$1.25. No. 73: Synopsis of the Tribe Salatini, with Description of Five New Genera and Three New Species (Pisces, Blenniidae). By William F. Smith-Vaniz and Victor G. Springer. Pp. 72. \$1.25. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) [65]

Geological Survey of Canada. Paper 69-56: A Collection of Papers prepared for a Study of the Earth Sciences in Canada, commissioned by the Science Council of Canada. Edited by C. H. Smith. Pp. iv+318. \$3.50. Paper 70-40: Basins and Geosynclines of the Canadian Shield. (A Collection of Papers presented at a Symposium held in Ottawa, March 17/18, 1970.) Edited by A. J. Baer. Pp. vi+165. \$3.50. Paper 70-64: The Adsorption and Coprecipitation of Silver on Hydrous Oxides of Iron and Manganese. By Willy Dyck. Pp. iii+23. \$1.50. Bulletin 180: Stratigraphy and Structure of the "Keno Hill Quartzite" in Tombstone River—Upper Klondike River May-Areas, Yukon Territory. By D. J. Tempelman-Kluit. Pp. 102. \$3. (Ottawa: Information Canada, 1970 and 1971.) [65]

The 1971 Eruption of Cerro Negro, Nicaragua. By J. G. Viramonte, E. Ubeda and M. Martinez. Pp. 28. (Cambridge, Mass.: Smithsonian Institution. Center for Short-Lived Phenomena, 1971.) [75]

Institut for Atomenergi, Kjeller Research Establishment. Kjeller Report No. 142: Instrumental Neutron Activation Analysis of Zircalloy Using a Ge(Li) Detector. By E. Steinnes. Pp. 11. (Kjeller, Norway: Institut for Atomenergi, Kjeller Research Establishment, 1971.) [115]

Role Playing and Teacher Education: a Manual for Developing Innovative Teachers. By David L. Lehman. Pp. 59. (Washington, DC: The Commission on Undergraduate Education in the Biological Sciences, 1971.) *gratis*. [115]

Pesticide Residues in Food: Report of the 1969 Joint Meeting of the FAO Working Party of Experts

on Pesticide Residues and the WHO Expert Group on Pesticide Residues, Rome, 8-15 December 1969. (FAO Agricultural Studies No. 84; World Health Organization Technical Report Series, No. 458.) Pp. vi+43. (Rome: FAO and WHO; London: HMSO, 1970.) [115]

Sveriges Geologiska Undersökning, Stockholm. Lovö Geomagnetic Observatory, Year Book 1968. Pp. 38. Lovö Geomagnetic Observatory, Year Book 1969. Pp. 38. (Stockholm: Sveriges Geologiska Undersökning, 1971.) [115]

Carnegie Institution of Washington, Year Book 1969, 1969-1970. Pp. x+767. (Washington, DC: Carnegie Institution of Washington, 1971.) [115]

Smithsonian Studies in History and Technology. No. 6: History of Letter Post Communication Between the United States and Europe, 1845-1875. By George E. Hargest. Pp. ix+234. (Washington, DC: Smithsonian Institution Press, 1971. For sale by US Government Printing Office.) \$4.25. [115]

Annals of the South African Museum. Vol. 57, Part 8: Amphipoda from Southern Angola. By Brian Kensly. Pp. 149-156. (Cape Town: South African Museum, 1971.) [125]

Annual Report of the Mauritius Institute for 1968. Pp. 12. Annual Report of the Mauritius Institute for 1969. Pp. 24. (Port Louis: Mauritius Institute, 1969 and 1970.) [135]

US Department of the Interior: Geological Survey. Professional Paper 599-F: Summary of Lunar Stratigraphy—Telescope Observations. By Don E. Wilhelms. Pp. iii+47. \$0.60. Professional Paper 638: Geology and Magnetite Deposits of the Franklin Quadrangle and Part of the Hamburg Quadrangle, New Jersey. By Donald R. Balser and A. F. Buddington. Pp. v+73+2 plates. Professional Paper 639: Ordovician Brachiopods, Trilobites, and Stratigraphy in Eastern and Central Nevada. By Reuben James Ross, Jr. Pp. iv+103+19 plates. Professional Paper 640-C: Eocene Mollusks from Eua, Tonga. By Harry S. Ladd. Pp. vii+10+5 plates. \$0.55. Professional Paper 644-B: Hydrocarbons in Thermal Areas, Northwestern Wyoming. By J. D. Love and John M. Good. Pp. 23. \$0.40. Water-Supply Paper 1904. Surface Water Supply of the United States 1961-65. Part 2: South Atlantic Slope and Eastern Gulf of Mexico Basins. Vol. 1: Basins from James River to Savannah River. Pp. x+942. (Washington, DC: Government Printing Office, 1970.) [135]

Canada: Department of Energy, Mines and Resources. Geological Survey of Canada. Bulletin No. 200: Biostratigraphy of some Early Middle Silurian Ostracoda, Eastern Canada. Additional Silurian Arthropoda from Arctic and Eastern Canada. By M. J. Copeland. Pp. 13+4 plates. \$1.50. Paper 70-12: Geology, Colville Lake Map-Area and Part of Coppermine Map-Area, Northwest Territories. By D. G. Cook and J. D. Aitken. Pp. vi+42. \$1.50. (Ottawa: Information Canada, 1971.) [145]

National Research Council of Canada. Annual Report on Support of University Research 1969/1970. Pp. viii+412. (Ottawa: National Research Council of Canada, 1971.) [185]

Australia: Commonwealth Scientific and Industrial Research Organization. Mineral Chemistry—Research Report 1969/1970. Pp. 20. (Port Melbourne: CSIRO, 1971.) [185]

US Department of the Interior: Geological Survey. Techniques of Water-Resources Investigations of the United States Geological Survey. Chapter A1: Methods for Collection and Analysis of Water Samples for Dissolved Minerals and Gases. By Eugene Brown, M. W. Skougstad and M. J. Fishman. (Book 5, Laboratory Analysis.) Pp. vii+160. (Washington, DC: Government Printing Office, 1970.) \$2. [185]

Bulletin of the American Museum of Natural History. Vol. 144, Article 1: Stratigraphy of the Santa Fe Group, New Mexico. By Ted Galusha and John C. Blick. Pp. 1-128. \$6. Vol. 144, Article 2: Gastropoda and Monoplacophora of the Solville Member (Middle Devonian, Marcellus Formation) in the Chenango Valley, New York State. By Harold B. Rollins, Niles Eldredge, and Judith Spiller. Pp. 129-170. \$2.30. (New York: American Museum of Natural History, 1971.) [185]

HOW TO BUY NATURE

Volumes start in January, March, May, July, September and November, but subscriptions may begin at any time.

The direct postal price per subscription is:

12 MONTHS* (52 issues per title)

	Surface Mail UK and worldwide	Airfreight U.S.A. Canada	
Nature (Friday)	£14	\$48	\$52
Nature+			
Nature Physical Science	£24	\$83	\$90
Nature+			
Nature New Biology	£24	\$83	\$90
All three editions	£29.50	\$108	\$116
Annual Index	£1	\$3	\$3

* Rates for shorter periods *pro rata* (minimum three months)

(Charge for delivery by air mail on application)

Editorial and Publishing Offices of NATURE

MACMILLAN JOURNALS LIMITED

4 LITTLE ESSEX STREET, LONDON WC2R 3LF

Telephone Number: 01-836 6633. Telegrams: Phusis London WC2R 3LF

711 NATIONAL PRESS BUILDING

WASHINGTON DC 20004

Telephone Number: 202-737 2355

Subscription Department

MACMILLAN JOURNALS LIMITED

BRUNEL ROAD, BASINGSTOKE, HANTS

Telephone Number: Basingstoke 5431

American display advertisements

NATURE SCIENTIFIC PUBLICATIONS INC

711 NATIONAL PRESS BUILDING

WASHINGTON DC 20004

All other advertisements

T. G. SCOTT & SON, LIMITED

1 CLEMENT'S INN, LONDON WC2A 2ED

Telephone: 01-242 6264/01-405 4743

Telegrams: Textualist London WC2A 2ED

Registered as a newspaper at the Post Office

Copyright © Macmillan Journals Limited, July 30 1971